METHOD AND APPARATUS FOR DEVICE
CONTROLLED GENE EXPRESSION

Field of the Invention

5   This invention relates generally to gene therapy of living tissue and particularly, but not by way of limitation, to method and apparatus for regulation of gene expression using a device generating gene transcription triggering signals.

Background of the Invention

10   The heart is the center of a person's circulatory system. It includes an electro-mechanical system performing two major pumping functions. The left portions of the heart draw oxygenated blood from the lungs and pump it to the organs of the body to provide the organs with their metabolic needs for oxygen. The right portions of the heart draw deoxygenated blood from the organs and pump

15   it into the lungs where the blood gets oxygenated. The body's metabolic need for oxygen increases with the body's physical activity level. The pumping functions are accomplished by contractions of the myocardium (heart muscles). In a normal heart, the sinoatrial node, the heart's natural pacemaker, generates electrical impulses, known as action potentials, that propagate through an electrical

20   conduction system to various regions of the heart to excite myocardial tissues in these regions. Coordinated delays in the propagations of the action potentials in a normal electrical conduction system cause the various regions of the heart to contract in synchrony such that the pumping functions are performed efficiently.

A blocked or otherwise damaged electrical conduction system causes the

25   myocardium to contract at a rhythm that is too slow, too fast, and/or irregular. Such an abnormal rhythm is generally known as arrhythmia. Arrhythmia reduces the heart's pumping efficiency and hence, diminishes the blood flow to the body. A deteriorated myocardium has decreased contractility, also resulting in diminished blood flow. A heart failure patient usually suffers from both a damaged electrical

30   conduction system and a deteriorated myocardium. The diminished blood flow results in insufficient blood supply to various body organs, preventing these organs

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to function properly and causing various symptoms. For example, in a patient suffering acute decompensated heart failure, an insufficient blood supply to the kidneys results in fluid retention and edema in the lungs and peripheral parts of the body, a condition referred to as decompensation. Without effective treatment, acute

5 decompensated heart failure cause rapid deterioration of the cardiovascular and general health and significantly shortened life expectancy. Treatments for arrhythmias and heart failure include, but are not limited to, electrical therapy such as pacing and defibrillation therapies, drug therapies, and biological therapies including gene-based therapies.

10 Gene-based therapies include the delivery of therapeutic genes to targeted cells and in some cases, the use of regulatable systems. For gene-based therapies which require expression of sequences in vectors, a promoter is linked to the sequence to be expressed. Strong viral promoters can drive a high level of expression in a wide range of tissues and cells, however, constitutive expression is

15 an open loop system and the encoded gene product may induce cellular toxicity or tolerance, or down regulation of expression through negative feedback.

One strategy to regulate the expression of target genes employs endogenous regulatable elements, and another strategy employs exogenous inducible gene expression systems. For example, heat-shock-induced loci have been used to

20 regulate the expression of a heterologous gene in mammalian cells (Wurm et al., Proc. Natl. Acad. Sci. USA, 83:5414 (1986); Bovenberg et al., Mol. Cell Endocrinol., 74:45 (1990)), and hypoxia-inducible *cis*-acting sequences from the erythropoietin gene allow a transcriptional response by hypoxia-inducible transcription factor (HIF-I) (Wang et al., Curr. Op. Hematol.,3:156 (1996)).

25 However, many regulatable systems based on endogenous promoters suffer from weak induction and high basal expression.

What is needed is a device useful to control expression of gene therapy vectors, e.g., to treat cardiovascular conditions.

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Summary of the Invention

The present invention provides spatial, temporal and/or conditional control of gene expression from one or more gene therapy vectors via an implantable device. The gene therapy vector includes one or more gene sequences useful to
5   alter, for instance, enhance or inhibit, expression of one or more native (endogenous) genes or the corresponding encoded gene product(s), or expression of one or more nonnative genes or the corresponding encoded gene product(s), in cells, which expression *in vivo* prevents, inhibits or treats at least one symptom of a particular condition. Thus, for a condition in which expression of a native gene is
10   aberrant, e.g., results in lack of or low expression of a functional gene product or overexpression of a functional gene product, or the native gene product lacks or has aberrant activity, the gene therapy vector includes an open reading frame for a gene operably linked to at least one regulatable transcriptional control element, the expression of which open reading frame in cells in an organism is effective to
15   prevent, inhibit or treat at least one symptom of a particular condition. In one embodiment, for conditions in which it is desirable to inhibit expression of a native gene or gene product, the gene therapy vector may include an appropriate antisense gene sequence or a mutant gene, e.g., one which encodes a dominant negative gene product, operably linked to at least one regulatable transcriptional control element.
20   In another embodiment, for conditions in which it is desirable to express or augment expression of a gene or gene product, the gene therapy vector may include an appropriate gene sequence or a portion thereof (sense orientation), i.e., a portion that encodes a gene product with substantially the same activity as the full length gene product, operably linked to at least one regulatable transcriptional control element.
25   In one embodiment, the condition is a cardiac condition and the expression of the gene(s) in the gene therapy vector in an animal, such as a mammal, having or at risk of the cardiac condition, alters the electrophysiologic properties in a defined region of the heart.

To control expression of the gene(s) in the gene therapy vector(s) once the
30   vector(s) are administered to an animal, an implantable device is employed. The device may be introduced to the animal before, concurrent with or after the gene

therapy vector(s) are administered. In one embodiment, the vector(s) which are delivered are not associated with an intact cell, e.g., a recombinant virus or isolated DNA having a desirable gene sequence is administered to the animal. In another embodiment, recombinant cells which include the gene therapy vector(s) are

5   employed, e.g., cells useful to express secreted proteins or useful in cell therapy. The implantable device includes a controller which emits a signal upon sensing a physiological parameter or a change in a physiological parameter, or as a result of an external command, the amount and/or strength of which signal alters expression, e.g., induces expression, of the gene that is operably linked to the regulatable

10   transcriptional control element in the gene therapy vector. Thus, the systems and methods of the invention which employ sensors and diagnostic information allow for control of gene therapy, thus providing for spatial, temporal and/or conditional dosing of the gene product encoded by the gene therapy vector(s) in an animal. Hence, titration of the dose administered is readily accomplished.

15   The invention thus provides a method to control expression of at least one exogenously introduced expression cassette which includes a regulatable transcriptional control element operably linked to an open reading frame in an animal at risk of a cardiac condition. The method includes providing an animal at risk of the cardiac condition which comprises a system of the invention,

20   e.g., a system which includes a sensor to sense a physiological signal indicative of a predetermined cardiac condition, a gene regulatory signal delivery device that emits a regulatory signal which directly or indirectly regulates a regulatable transcriptional control element, and a controller coupled to the sensor and the gene regulatory signal delivery device, the controller adapted to control the emission of the

25   regulatory signal based on at least the sensed physiological signal. At least one expression cassette that is introduced to the animal includes a transcriptional control element, which is directly or indirectly regulated by the emitted signal, operably linked to an open reading frame. In response to detection of the condition, a signal is emitted from the system so as to control expression of the open reading frame.

30   The expression of the open reading frame in the animal prevents, inhibits or treats the condition or at least one symptom thereof.

Also provided is a method to control expression of at least one exogenously introduced expression cassette which includes a regulatable transcriptional control element operably linked to an open reading frame in an animal at risk of a cardiac condition. The method includes providing an animal comprising at least one

5   exogenously introduced expression cassette that includes a transcriptional control element, which is directly or indirectly regulated by a signal, operably linked to an open reading frame, the expression of which is capable of preventing, inhibiting or treating a cardiac condition or at least one symptom thereof in an animal at risk of the cardiac condition. A system of the invention is introduced to the animal, and

10   in response to detection of the condition, a signal is emitted from the system so as to control expression of the open reading frame. The expression of the open reading frame in the animal prevents, inhibits or treats the condition or at least one symptom thereof.

In one embodiment, the invention provides a method to prepare an

15   implantable device effective to control expression of at least one exogenously introduced expression cassette which includes a regulatable transcriptional control element operably linked to an open reading frame in an animal at risk of a cardiac condition. The method includes introducing to an implantable device a gene regulatory signal delivery device that emits a regulatory signal which directly or

20   indirectly regulates the transcriptional control element. The expression of the open reading frame in the at least one exogenously introduced expression cassette is capable of preventing, inhibiting or treating the condition or at least one symptom thereof.

In one embodiment, a mammal has heart failure and is contacted with

25   a gene therapy vector having a Serca2A gene operably linked to a phytochrome promoter. The mammal is also contacted with an implantable device which detects decreased cardiac function, e.g., decreased heart rate variability (HRV). Upon detection of decreased cardiac function, the implantable device emits light which induces expression from the phytochrome promoter. For instance, the device emits

30   an appropriate signal via a fiber optic or LED. The light signal is emitted so as to express the Serca2A gene in the gene therapy vector in an amount effective to

enhance cardiac function. In another embodiment, a mammal has heart failure and is contacted with a gene therapy vector having a β-adrenergic signaling protein gene operably linked to a promoter sensitive to a subthreshold (below threshold for muscular excitation), threshold, or suprathreshold voltage, or a combination thereof,

5   e.g., a MT-1 promoter. The mammal is also contacted with an implantable device which detects decreased cardiac function. Upon detection of decreased cardiac function, the implantable device emits a voltage which induces expression from the promoter. The signal is emitted so as to express the β-adrenergic signaling protein gene in the gene therapy vector in an amount effective to enhance cardiac function.

10   In one embodiment, the signal emitted from the device induces expression of the gene and provides for electrical therapy, e.g., pacing or defibrillation. In yet another embodiment, a mammal suffers from atrial fibrillation and is contacted with a gene therapy vector having a Kir2.1 gene operably linked to a thermal sensitive promoter. The mammal is contacted with an implantable device which detects an atrial

15   electrogram. Upon detection of an atrial arrhythmia, the implantable device emits heat and Kir2.1 is expressed from the gene therapy vector in an amount effective to terminate atrial fibrillation. For example, once sinus rhythm is restored, the heat signal emitted from the device is terminated. Thus, the systems of the invention provide for feedback control of gene therapy.

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Brief Description of the Drawings

The drawings illustrate generally, by way of example, but not by way of limitation, various embodiments discussed in the present document. The drawing are for illustrative purposes only and are not drawn to scale.

25   FIG. 1 is an illustration of an embodiment of a gene regulatory system and portions of an environment in which it is used.

FIG. 2 is a block diagram showing one embodiment of a circuit of portions of the gene regulatory system such as shown in FIG. 1.

FIG. 3 is a block diagram showing another embodiment of the circuit of

30   portions of the gene regulatory system such as shown in FIG. 1.

Detailed Description of the Invention

In the following detailed description, reference is made to the accompanying drawings which form a part hereof, and in which is shown by way of illustration specific embodiments in which the invention may be practiced. These embodiments are described in sufficient detail to enable those skilled in the art to practice the invention, and it is to be understood that the embodiments may be combined, or that other embodiments may be utilized and that structural, logical and electrical changes may be made without departing from the spirit and scope of the present invention. The following detailed description provides examples, and the scope of the present invention is defined by the appended claims and their equivalents.

It should be noted that references to "an", "one", or "various" embodiments in this disclosure are not necessarily to the same embodiment, and such references contemplate more than one embodiment.

General Overview

This document describes, among other things, methods and apparatus for the control of gene therapy. In one embodiment, a mammal having or at risk of having a particular condition, e.g., a cardiovascular condition, is subjected to gene therapy which is intended to inhibit, prevent or treat one or more symptoms associated with the condition. The gene therapy vector encodes at least one therapeutic gene product and is operably linked to at least one regulatable transcriptional control element, forming an expression cassette. In one embodiment, the gene therapy vector includes at least one transgene that encodes a gene product including, but not limited to, an angiogenic protein, a growth factor, a differentiation factor, a survival factor, a cytokine, a cardiac cell-specific structural gene product, a cardiac cell-specific transcription factor, or a membrane protein, e.g., a gap junction or an ion channel protein, or including an antisense sequence, for instance, a ribozyme or antisense oligonucleotide, or any combination thereof. The expression of the gene product is under the control of a regulatable transcriptional control element such as a promoter, e.g., an inducible or repressible promoter, or an enhancer. For instance, the enhancer may be a glucocorticoid responsive enhancer or the promoter may be an electromagnetic responsive promoter. In one embodiment, the expression of the

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gene is also disease-, cell- or tissue-specific, e.g., cardiac cell-specific, due to a disease-, cell- or tissue-specific promoter and/or enhancer. For instance, the enhancer may be a muscle creatine kinase (mck) enhancer, or the promoter may be an alpha-myosin heavy chain (MyHC) or beta-MyHC promoter (see Palermo et al.,

5   Circ. Res., 78, 504 (1996)). In one embodiment, the transcriptional regulatory element is upregulated by certain disease states, e.g., to treat heart failure or left bundle blockage, a vector of the invention may include a transcriptional regulatory element from a gene that is upregulated in patients having heart failure or upregulated during progression of heart failure, e.g., transcriptional regulatory

10   elements from genes including but not limited to those for an extracellular signal-regulated kinase (ERK), mitogen-activated protein kinase (MAPK), stress activated protein kinase (SAPK), p38, calcineurin, Akt, Na^+/Ca^{2+} exchanger (NCX), metal metalloproteinase-2 (MMP-2) or MMP-7. To treat atrial fibrillation, a vector of the invention may include a transcriptional regulatory element from a gene that is

15   upregulated in patients having atrial fibrillation, e.g., genes associated with the production of reactive oxygen species (ROS) including flavin containing monooxygenase I, monoamine oxidase B, ubiquitin specific protease 8, tyrosinase-related protein 1, tyrosine 3 related monoxygenase, MMP-2 or MMP-7. To treat systolic dysfunction, a vector of the invention may include a transcriptional

20   regulatory element from a gene such as the one encoding the Na^+/Ca^{2+} exchanger. Optionally, a combination of gene therapy vectors, each with a different transgene and at least one of which includes a regulatable transcriptional control element, is employed.

Prior to, concurrent with or after gene therapy, an implantable device which

25   regulates expression of the gene(s) in the gene therapy vector is provided to the animal. In one embodiment, the device is introduced at or near damaged cardiovascular tissue. In response to detection of a symptom of a condition, e.g., a change in a physiological parameter such as heart rate, the device emits a signal which activates the regulatable transcriptional control element in the gene therapy

30   vector. Such signals include, but are not limited to, an electric field, electromagnetic field, light, sound, temperature, and/or chemical agents such as a

biologic agent (i.e., one encoded by DNA) or a nonbiologic agent, e.g., a beta adrenergic blocker, an alpha adrenergic blocker, a calcium channel blocker, an ACE inhibitor or an angiotension II blocker. In one embodiment, after expression from the gene therapy vector is induced and a desirable change in the physiological

5   parameter detected, the signal is discontinued. In another embodiment, the signal is emitted for a predetermined time period. Thus, gene expression may be turned on and off or titrated by controlling signals emitted by the device.

Thus, this document discusses a gene regulatory system that includes an implantable gene regulatory signal delivery device being a portion of, or being

10   coupled to, an implantable medical device. In one embodiment, the implantable medical device detects a predetermined condition indicative of a need for a therapy. In response, the implantable gene regulatory signal delivery device delivers one or more signals. In one further embodiment, the gene regulatory therapy is performed in conjunction with electrical therapy, such as pacing therapy, and/or drug therapy.

15   One specific example of the implantable medical device is an implantable cardiac rhythm management (CRM) device. Though discussed specifically in this document as part of a CRM system, the gene regulatory system is generally usable for all *in vivo* gene therapies. Several embodiments are presented below to provide examples of different therapy apparatus and method.

20   Definitions

A "vector" or "construct" (sometimes referred to as gene delivery or gene transfer "vehicle") refers to a macromolecule or complex of molecules comprising a polynucleotide to be delivered to a host cell, either *in vitro* or *in vivo*. The polynucleotide to be delivered may comprise a sequence of interest for gene

25   therapy. Vectors include, for example, transposons and other site-specific mobile elements, viral vectors, e.g., adenovirus, adeno-associated virus (AAV), poxvirus, papillomavirus, lentivirus, herpesvirus, foamivirus and retrovirus vectors, and including pseudotyped viruses, liposomes and other lipid-containing complexes, and other macromolecular complexes capable of mediating delivery of a polynucleotide

30   to a host cell, e.g., DNA coated gold particles, polymer-DNA complexes, liposome-DNA complexes, liposome-polymer-DNA complexes, virus-polymer-DNA

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complexes, e.g., adenovirus-polylysine-DNA complexes, and antibody-DNA complexes. Vectors can also comprise other components or functionalities that further modulate gene delivery and/or gene expression, or that otherwise provide beneficial properties to the cells to which the vectors will be introduced. Such other

5   components include, for example, components that influence binding or targeting to cells (including components that mediate cell-type or tissue-specific binding); components that influence uptake of the vector nucleic acid by the cell; components that influence localization of the polynucleotide within the cell after uptake (such as agents mediating nuclear localization); and components that influence expression of

10   the polynucleotide. Such components also might include markers, such as detectable and/or selectable markers that can be used to detect or select for cells that have taken up and are expressing the nucleic acid delivered by the vector. Such components can be provided as a natural feature of the vector (such as the use of certain viral vectors which have components or functionalities mediating binding

15   and uptake), or vectors can be modified to provide such functionalities. A large variety of such vectors are known in the art and are generally available. When a vector is maintained in a host cell, the vector can either be stably replicated by the cells during mitosis as an autonomous structure, incorporated within the genome of the host cell, or maintained in the host cell's nucleus or cytoplasm.

20   A "recombinant viral vector" refers to a viral vector comprising one or more heterologous genes or sequences. Since many viral vectors exhibit size constraints associated with packaging, the heterologous genes or sequences are typically introduced by replacing one or more portions of the viral genome. Such viruses may become replication-defective, requiring the deleted function(s) to be provided in

25   *trans* during viral replication and encapsidation (by using, e.g., a helper virus or a packaging cell line carrying genes necessary for replication and/or encapsidation). Modified viral vectors in which a polynucleotide to be delivered is carried on the outside of the viral particle have also been described (see, e.g., Curiel et al., Proc. Natl. Acad. Sci. USA, 88:8850 (1991)).

30   "Gene delivery," "gene transfer," and the like as used herein, are terms referring to the introduction of an exogenous polynucleotide (sometimes referred to

as a "transgene") into a host cell, irrespective of the method used for the introduction. Such methods include a variety of well-known techniques such as vector-mediated gene transfer (by, e.g., viral infection/transfection, or various other protein-based or lipid-based gene delivery complexes) as well as techniques

5   facilitating the delivery of "naked" polynucleotides (such as electroporation, iontophoresis, "gene gun" delivery and various other techniques used for the introduction of polynucleotides). The introduced polynucleotide may be stably or transiently maintained in the host cell. Stable maintenance typically requires that the introduced polynucleotide either contains an origin of replication compatible with

10   the host cell or integrates into a replicon of the host cell such as an extrachromosomal replicon (e.g., a plasmid) or a nuclear or mitochondrial chromosome. A number of vectors are known to be capable of mediating transfer of genes to mammalian cells, as is known in the art.

By "transgene" is meant any piece of a nucleic acid molecule (for example,

15   DNA) which is inserted by artifice into a cell either transiently or permanently, and becomes part of the organism if integrated into the genome or maintained extrachromosomally. Such a transgene may include a gene which is partly or entirely heterologous (i.e., foreign) to the transgenic organism, or may represent a gene homologous to an endogenous gene of the organism.

20   By "transgenic cell" is meant a cell containing a transgene. For example, a stem cell transformed with a vector containing an expression cassette can be used to produce a population of cells having altered phenotypic characteristics.

The term "wild-type" refers to a gene or gene product that has the characteristics of that gene or gene product when isolated from a naturally occurring

25   source. A wild-type gene is that which is most frequently observed in a population and is thus arbitrarily designated the "normal" or "wild-type" form of the gene. In contrast, the term "modified" or "mutant" refers to a gene or gene product that displays modifications in sequence and or functional properties (i.e., altered characteristics) when compared to the wild-type gene or gene product. It is noted

30   that naturally-occurring mutants can be isolated; these are identified by the fact that

they have altered characteristics when compared to the wild-type gene or gene product.

A "disease allele" refers to an allele of a gene that is capable of producing a recognizable disease. However, not all conditions or populations having a certain condition have a known disease allele. A disease allele may be dominant or recessive and may produce disease directly or when present in combination with a specific genetic background or pre-existing pathological condition. A disease allele may be present in the gene pool (an inherited disease allele) or may be generated *de novo* in an individual by somatic mutation (an acquired disease allele).

"Vasculature" or "vascular" are terms referring to the system of vessels carrying blood (as well as lymph fluids) throughout the mammalian body.

"Blood vessel" refers to any of the vessels of the mammalian vascular system, including arteries, arterioles, capillaries, venules, veins, sinuses, and vasa vasorum.

"Artery" refers to a blood vessel through which blood passes away from the heart. Coronary arteries supply the tissues of the heart itself, while other arteries supply the remaining organs of the body. The general structure of an artery consists of a lumen surrounded by a multi-layered arterial wall.

The term "transduction" denotes the delivery of a polynucleotide to a recipient cell either *in vivo* or *in vitro*, via a viral vector and preferably via a replication-defective viral vector, such as via a recombinant AAV.

The term "heterologous" as it relates to nucleic acid sequences such as gene sequences and control sequences, denotes sequences that are not normally joined together, and/or are not normally associated with a particular cell. Thus, a "heterologous" region of a nucleic acid construct or a vector is a segment of nucleic acid within or attached to another nucleic acid molecule that is not found in association with the other molecule in nature. For example, a heterologous region of a nucleic acid construct could include a coding sequence flanked by sequences not found in association with the coding sequence in nature, i.e., a heterologous promoter. Another example of a heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., synthetic sequences having

codons different from the native gene). Similarly, a cell transformed with a construct which is not normally present in the cell would be considered heterologous for purposes of this invention.

By "DNA" is meant a polymeric form of deoxyribonucleotides (adenine,

5   guanine, thymine, or cytosine) in double-stranded or single-stranded form found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA

10 (i.e., the strand having the sequence complementary to the mRNA). The term captures molecules that include the four bases adenine, guanine, thymine, or cytosine, as well as molecules that include base analogues which are known in the art.

As used herein, the terms "complementary" or "complementarity" are used

15 in reference to polynucleotides (i.e., a sequence of nucleotides) related by the base-pairing rules. For example, the sequence "A-G-T," is complementary to the sequence "T-C-A." Complementarity may be "partial," in which only some of the nucleic acids' bases are matched according to the base pairing rules. Or, there may be "complete" or "total" complementarity between the nucleic acids. The degree of

20 complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands. This is of particular importance in amplification reactions, as well as detection methods that depend upon binding between nucleic acids.

DNA molecules are said to have "5' ends" and "3' ends" because

25 mononucleotides are reacted to make oligonucleotides or polynucleotides in a manner such that the 5' phosphate of one mononucleotide pentose ring is attached to the 3' oxygen of its neighbor in one direction via a phosphodiester linkage. Therefore, an end of an oligonucleotide or polynucleotide is referred to as the "5' end" if its 5' phosphate is not linked to the 3' oxygen of a mononucleotide pentose

30 ring and as the "3' end" if its 3' oxygen is not linked to a 5' phosphate of a subsequent mononucleotide pentose ring. As used herein, a nucleic acid sequence,

even if internal to a larger oligonucleotide or polynucleotide, also may be said to have 5' and 3' ends. In either a linear or circular DNA molecule, discrete elements are referred to as being "upstream" or 5' of the "downstream" or 3' elements. This terminology reflects the fact that transcription proceeds in a 5' to 3' fashion along

5   the DNA strand. The promoter and enhancer elements that direct transcription of a linked gene are generally located 5' or upstream of the coding region. However, enhancer elements can exert their effect even when located 3' of the promoter element and the coding region. Transcription termination and polyadenylation signals are located 3' or downstream of the coding region.

10   A "gene," "polynucleotide," "coding region," or "sequence" which "encodes" a particular gene product, is a nucleic acid molecule which is transcribed and optionally also translated into a gene product, e.g., a polypeptide, *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences. The coding region may be present in either a cDNA, genomic DNA, or RNA form. When

15   present in a DNA form, the nucleic acid molecule may be single-stranded (i.e., the sense strand) or double-stranded. The boundaries of a coding region are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. A gene can include, but is not limited to, cDNA from prokaryotic or eukaryotic mRNA, genomic DNA sequences from prokaryotic or

20   eukaryotic DNA, and synthetic DNA sequences. Thus, a gene includes a polynucleotide which may include a full-length open reading frame which encodes a gene product (sense orientation) or a portion thereof (sense orientation) which encodes a gene product with substantially the same activity as the gene product encoded by the full-length open reading frame, the complement of the

25   polynucleotide, e.g., the complement of the full-length open reading frame (antisense orientation) and optionally linked 5' and/or 3' noncoding sequence(s) or a portion thereof, e.g., an oligonucleotide, which is useful to inhibit transcription, stability or translation of a corresponding mRNA. A transcription termination sequence will usually be located 3' to the gene sequence.

30   An "oligonucleotide" includes at least 7 nucleotides, preferably 15, and more preferably 20 or more sequential nucleotides, up to 100 nucleotides, either RNA or

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DNA, which correspond to the complement of the non-coding strand, or of the coding strand, of a selected mRNA, or which hybridize to the mRNA or DNA encoding the mRNA and remain stably bound under moderately stringent or highly stringent conditions, as defined by methods well known to the art, e.g., in Sambrook

5   et al., A Laboratory Manual, Cold Spring Harbor Press (1989).

The term "control elements" refers collectively to promoter regions, polyadenylation signals, transcription termination sequences, upstream regulatory domains, origins of replication, internal ribosome entry sites ("IRES"), enhancers, splice junctions, and the like, which collectively provide for the replication,

10   transcription, post-transcriptional processing and translation of a coding sequence in a recipient cell. Not all of these control elements need always be present so long as the selected coding sequence is capable of being replicated, transcribed and translated in an appropriate host cell.

The term "promoter region" is used herein in its ordinary sense to refer to a

15   nucleotide region comprising a DNA regulatory sequence, wherein the regulatory sequence is derived from a gene which is capable of binding RNA polymerase and initiating transcription of a downstream (3' direction) coding sequence. Thus, a "promoter," refers to a polynucleotide sequence that controls transcription of a gene or coding sequence to which it is operably linked. A large number of promoters,

20   including constitutive, inducible and repressible promoters, from a variety of different sources, are well known in the art.

By "enhancer element" is meant a nucleic acid sequence that, when positioned proximate to a promoter, confers increased transcription activity relative to the transcription activity resulting from the promoter in the absence of the

25   enhancer domain. Hence, an "enhancer" includes a polynucleotide sequence that enhances transcription of a gene or coding sequence to which it is operably linked. A large number of enhancers, from a variety of different sources are well known in the art. A number of polynucleotides which have promoter sequences (such as the commonly-used CMV promoter) also have enhancer sequences.

30   By "cardiac-specific enhancer or promoter" is meant an element, which, when operably linked to a promoter or alone, respectively, directs gene expression

in a cardiac cell and does not direct gene expression in all tissues or all cell types. Cardiac-specific enhancers or promoters may be naturally occurring or non-naturally occurring. One skilled in the art will recognize that the synthesis of non-naturally occurring enhancers or promoters can be performed using standard

5   oligonucleotide synthesis techniques.

"Operably linked" refers to a juxtaposition, wherein the components so described are in a relationship permitting them to function in their intended manner. By "operably linked" with reference to nucleic acid molecules is meant that two or more nucleic acid molecules (e.g., a nucleic acid molecule to be transcribed, a

10   promoter, and an enhancer element) are connected in such a way as to permit transcription of the nucleic acid molecule. A promoter is operably linked to a coding sequence if the promoter controls transcription of the coding sequence. Although an operably linked promoter is generally located upstream of the coding sequence, it is not necessarily contiguous with it. An enhancer is operably linked to a coding

15   sequence if the enhancer increases transcription of the coding sequence. Operably linked enhancers can be located upstream, within or downstream of coding sequences. A polyadenylation sequence is operably linked to a coding sequence if it is located at the downstream end of the coding sequence such that transcription proceeds through the coding sequence into the polyadenylation sequence.

20   "Operably linked" with reference to peptide and/or polypeptide molecules is meant that two or more peptide and/or polypeptide molecules are connected in such a way as to yield a single polypeptide chain, i.e., a fusion polypeptide, having at least one property of each peptide and/or polypeptide component of the fusion. Thus, a signal or targeting peptide sequence is operably linked to another protein if the resulting

25   fusion is secreted from a cell as a result of the presence of a secretory signal peptide or into an organelle as a result of the presence of an organelle targeting peptide.

"Homology" refers to the percent of identity between two polynucleotides or two polypeptides. The correspondence between one sequence and to another can be determined by techniques known in the art. For example, homology can be

30   determined by a direct comparison of the sequence information between two polypeptide molecules by aligning the sequence information and using readily

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available computer programs. Alternatively, homology can be determined by hybridization of polynucleotides under conditions which form stable duplexes between homologous regions, followed by digestion with single strand-specific nuclease(s), and size determination of the digested fragments. Two DNA, or two

5 polypeptide, sequences are "substantially homologous" to each other when at least about 80%, preferably at least about 90%, and most preferably at least about 95% of the nucleotides, or amino acids, respectively match over a defined length of the molecules, as determined using the methods above.

By "mammal" is meant any member of the class *Mammalia* including,

10 without limitation, humans and nonhuman primates such as chimpanzees and other apes and monkey species; farm animals such as cattle, sheep, pigs, goats and horses; domestic mammals such as dogs and cats; laboratory animals including rodents such as mice, rats, rabbits and guinea pigs, and the like. An "animal" includes vertebrates such as mammals, avians, amphibians, reptiles and aquatic organisms

15 including fish.

By "derived from" is meant that a nucleic acid molecule was either made or designed from a parent nucleic acid molecule, the derivative retaining substantially the same functional features of the parent nucleic acid molecule, e.g., encoding a gene product with substantially the same activity as the gene product encoded by the

20 parent nucleic acid molecule from which it was made or designed.

By "expression construct" or "expression cassette" is meant a nucleic acid molecule that is capable of directing transcription. An expression construct includes, at the least, a promoter. Additional elements, such as an enhancer, and/or a transcription termination signal, may also be included.

25 The term "exogenous," when used in relation to a protein, gene, nucleic acid, or polynucleotide in a cell or organism refers to a protein, gene, nucleic acid, or polynucleotide which has been introduced into the cell or organism by artificial or natural means, or in relation a cell refers to a cell which was isolated and subsequently introduced to other cells or to an organism by artificial or natural

30 means. An exogenous nucleic acid may be from a different organism or cell, or it may be one or more additional copies of a nucleic acid which occurs naturally

within the organism or cell. An exogenous cell may be from a different organism, or it may be from the same organism. By way of a non-limiting example, an exogenous nucleic acid is in a chromosomal location different from that of natural cells, or is otherwise flanked by a different nucleic acid sequence than that found in

5   nature.

The term "isolated" when used in relation to a nucleic acid, peptide, polypeptide or virus refers to a nucleic acid sequence, peptide, polypeptide or virus that is identified and separated from at least one contaminant nucleic acid, polypeptide, virus or other biological component with which it is ordinarily

10   associated in its natural source. Isolated nucleic acid, peptide, polypeptide or virus is present in a form or setting that is different from that in which it is found in nature. For example, a given DNA sequence (e.g., a gene) is found on the host cell chromosome in proximity to neighboring genes; RNA sequences, such as a specific mRNA sequence encoding a specific protein, are found in the cell as a mixture with

15   numerous other mRNAs that encode a multitude of proteins. The isolated nucleic acid molecule may be present in single-stranded or double-stranded form. When an isolated nucleic acid molecule is to be utilized to express a protein, the molecule will contain at a minimum the sense or coding strand (i.e., the molecule may single-stranded), but may contain both the sense and anti-sense strands (i.e., the molecule

20   may be double-stranded).

The term "recombinant DNA molecule" as used herein refers to a DNA molecule that is comprised of segments of DNA joined together by means of molecular biological techniques.

The term "recombinant protein" or "recombinant polypeptide" as used herein

25   refers to a protein molecule that is expressed from a recombinant DNA molecule.

The term "peptide", "polypeptide" and protein" are used interchangeably herein unless otherwise distinguished to refer to polymers of amino acids of any length. These terms also include proteins that are post-translationally modified through reactions that include glycosylation, acetylation and phosphorylation.

30   By "growth factor" is meant an agent that, at least, promotes cell growth or induces phenotypic changes.

The term "angiogenic" means an agent that alone or in combination with other agents induces angiogenesis, and includes, but is not limited to, fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), hepatocyte growth factor, angiogenin, transforming growth factor (TGF), tissue necrosis factor (TNF,

5 e.g., TNF-α), platelet derived growth factor (PDGF), granulocyte colony stimulatory factor (GCSF), placental GF, IL-8, proliferin, angiopoietin, e.g., angiopoietin-1 and angiopoietin-2, thrombospondin, ephrin-A1, E-selectin, leptin and heparin affinity regulatory peptide.

"Gene regulation" or "Gene regulatory therapy" as used herein includes

10 delivery of one or more gene regulatory signals to regulate gene expression in a gene therapy vector. The gene regulatory signals include signals that trigger a transcriptional control element, e.g., a promoter.

A "user" includes a physician or other caregiver using a gene regulatory system to treat a patient.

15 Apparatus

FIG. 1 is an illustration of an embodiment of a gene regulatory system 100 and portions of an environment in which it is used. System 100 includes implantable system 105, external system 155, and telemetry link 140 providing for communication between implantable system 105 and external system 155.

20 Implantable system 105 includes, among other things, implantable CRM device 110, lead system 108, and implantable gene regulatory signal delivery device 130. As shown in FIG. 1, implantable CRM device 110 is implanted in a body 102. In one embodiment, implantable CRM device 110 includes a gene regulatory controller. In one embodiment, implantable gene regulatory signal delivery device

25 130 delivers one or more gene regulatory signals to heart 101. In another embodiment, implantable gene regulatory signal delivery device 130 delivers one or more gene regulatory signals to the vascular system of body 102, such as a vein. In various other embodiments, implantable gene regulatory signal delivery device 130 delivers one or more gene regulatory signals to any site within body 102 targeted for

30 the gene regulatory therapy. Lead system 108 provides for access to one or more locations to which the regulatory one or more gene regulatory signals are delivered.

In one embodiment, lead system 108 includes one or more leads providing for electrical connections between implantable CRM device 110 and implantable gene regulatory signal delivery device 130. In another embodiment, lead system 108 provides for the transmission of the one or more gene regulatory signals to the

5 locations to which the signals are delivered. In various embodiments, implantable CRM device 110 also includes a pacemaker, a cardioverter/defibrillator, a cardiac resynchronization therapy (CRT) device, a cardiac remodeling control therapy (RCT) device, a drug delivery device or a drug delivery controller, a cell therapy device, or any other implantable medical device. Lead system 108 further includes

10 leads for sensing physiological signals and delivering pacing pulses, cardioversion/defibrillation shocks, and/or pharmaceutical or other substances.

External system 155 includes external device 150, network 160, and remote device 170. External device 150 is within the vicinity of implantable CRM device 110 and communicates with implantable CRM device 110 bi-directionally via

15 telemetry link 140. Remote device 170 is in a remote location and communicates with external device 150 bi-directionally via network 160, thus allowing a user to monitor and treat a patient from a distant location.

System 100 allows a delivery of a gene regulatory therapy, i.e., delivery of the one or more gene regulatory signals, to be triggered by any one of implantable

20 CRM device 110, external device 150, and remote device 170. In one embodiment, implantable CRM device 110 triggers the delivery of the gene regulatory therapy upon detecting a predetermined signal or condition. In another embodiment, external device 150 or remote device 170 triggers the delivery of the gene regulatory therapy upon detecting an abnormal condition from a signal transmitted

25 from implantable CRM device 110. In one specific embodiment, external system 155 includes a processor running a therapy decision algorithm to determine whether and when to trigger the delivery of the gene regulatory therapy. In another specific embodiment, external system 155 includes a user interface to present signals acquired by implantable CRM device 155 and/or the detected abnormal condition to

30 a user and receives commands from the user for triggering the delivery of the gene regulatory therapy. In another specific embodiment, the user interface includes a

user input incorporated into external device 150 to receive commands from the user and/or the patient treated with system 100. For example, the patient may be instructed to enter a command for the gene regulatory therapy when he senses certain symptoms, and another person near the patient may do the same upon

5   observing the symptoms.

It is to be understood that an implantable gene regulatory signal delivery device and an implantable CRM device are discussed to illustrate, but not to restrict, the present subject matter. Though discussed specifically as part of a CRM system, the gene regulatory system and method discussed in this document is generally

10   usable for all *in vivo* gene therapies delivered by implantable or external devices.

FIG. 2 is a block diagram showing one embodiment of the circuit of portions of system 100 including implantable CRM device 110A, lead system 108, and implantable gene regulatory signal delivery device 130. Implantable CRM device 110A represents one specific embodiment of implantable CRM device 110. In one

15   embodiment, lead system 108 provides for an electrical connection between implantable CRM device 110A and implantable gene regulatory signal delivery device 130, such that implantable CRM device transmits a voltage or current signal to control the delivery of a gene regulatory signal.

Implantable gene regulatory signal delivery device 130 receives a gene

20   regulatory control signal from implant controller 214 and, in response, delivers one or more gene regulatory signals in one or more forms of energy being external factors regulating one or more gene expressions. The forms of energy include electrical energy, electromagnetic energy, optical energy, acoustic energy, thermal energy, and any other forms of energy that triggers the gene promoter system. In

25   one embodiment, implantable gene regulatory signal delivery device 130 delivers the one or more gene regulatory signals to the heart. In one specific embodiment, implantable gene regulatory signal delivery device 130 is an implantable device designed for placement within the heart. In another embodiment, implantable gene regulatory signal delivery device 130 delivers the one or more gene regulatory

30   signals to the blood. In one specific embodiment, implantable gene regulatory

signal delivery device 130 is an implantable device designed for placement within with the vascular system, such as in a vein.

In one embodiment, implantable gene regulatory signal delivery device 130 includes an electric field generator that generates and emits an electric field. The

5   electric field has predetermined frequency and strength selected for regulating gene expression in an exogenously introduced vector. In one specific embodiment, an electric field generator includes electrodes to which a voltage is applied. The intensity of the electric field is controlled by controlling the voltage across the electrodes.

10   In one embodiment, implantable gene regulatory signal delivery device 130 includes an electromagnetic field generator that generates and emits an electromagnetic field. The electromagnetic field has predetermined frequency and strength selected for regulating gene expression in an exogenously introduced vector. In one specific embodiment, the electromagnetic field generator includes an

15   inductive coil. The intensity of the electromagnetic field is controlled by controlling the voltage across the coil and/or the current flowing through it. In one specific embodiment, the electromagnetic field has a frequency of about 1 Hz to 1 KHz. In another specific embodiment, the electromagnetic field is a direct-current (dc) electromagnetic field.

20   In one embodiment, implantable gene regulatory signal delivery device 130 includes an optical emitter that emits light. The light has predetermined wavelength or band of wavelengths and intensity selected for regulating gene expression. In one specific embodiment, the optical emitter includes a light-emitting diode (LED). The intensity of the light is controlled by controlling the voltage across the LED and/or

25   the current flowing through it. In another specific embodiment, the optical emitter includes an array of LEDs that can be programmed to emit lights having one or more distinct wavelengths.

In one embodiment, implantable gene regulatory signal delivery device 130 includes a speaker that emits a sound. The sound has a predetermined frequency

30   and intensity selected for regulating gene expression in an exogenously introduced vector.

In one embodiment, implantable gene regulatory signal delivery device 130 includes a drug delivery device which emits one or more chemical agents. The one or more chemical agents have properties known to regulate expression from a transcriptional control element. Examples of the one or more chemical agents

5   include chemicals which induce expression from a particular promoter, including tetracycline, rapamycin, auxins, metals and ecdysone.

In one embodiment, implantable gene regulatory signal delivery device 130 includes a thermal radiator that emits a thermal energy. The thermal energy changes the tissue temperature to a point or range suitable for regulating gene expression in

10   an exogenously introduced vector. In one specific embodiment, the thermal radiator includes a resistive element that is heated as an electrical current flows through it or as a voltage is applied across it. The tissue temperature is controlled by controlling the amplitude of the electrical current or voltage.

Implantable CRM device 110A includes sensor 212, event detector 213, and

15   implant controller 214. Sensor 212 senses a physiological signal indicative of an abnormal condition treatable by a gene regulatory therapy administrated through implantable gene regulatory signal delivery device 130. Event detector 213 detects that abnormal condition. Implant controller 214 produces and transmits the gene regulatory control signal to implantable gene regulatory signal delivery device 130

20   to trigger a delivery of the gene regulatory therapy in response to a detected abnormal condition. In one embodiment, the gene regulatory therapy is delivered for a predetermined period of delivery time. In one specific embodiment, implant controller 214 includes a timer to time the predetermined period of delivery time and produces and transmits a gene regulatory stop signal to implantable gene

25   regulatory signal delivery device 130 to stop the delivery of the gene regulatory therapy. In another specific embodiment, implantable gene regulatory signal delivery device 130 includes a timer to time the predetermined period of delivery time and stops the delivery of the gene regulatory therapy when the predetermined period of delivery time expires. In another embodiment, implant controller 214

30   produces and transmits the gene regulatory stop signal to implantable gene regulatory signal delivery device 130 to stop the delivery of the gene regulatory

therapy after the abnormal condition is no longer detected by event detector 213. In another embodiment, implant controller 214 produces and transmits the gene regulatory stop signal to implantable gene regulatory signal delivery device 130 to stop the delivery of the gene regulatory therapy in response to a command from the user or patient. In one embodiment, event detector 213 further comprising an event parameter generator to produce one or more parameters related to at least one of a type and a degree of the abnormal condition. Implant controller 214 includes a regulatory signal parameter controller to quantitatively control the emission of the regulatory signal based the one or more parameters produced by the event parameter generator. In this embodiment, the gene regulatory control signal includes parameters defining the gene regulatory therapy to be delivered. The parameters include type(s) and quantitative parameters, depending on the type(s), such as electric field strength, electromagnetic field strength and frequency, light intensity and wavelength, sound intensity and frequency, type and amount of chemical, and/or amount of thermal energy.

In one embodiment, sensor 212 includes a cardiac sensing circuit that senses an electrogram, and event detector 213 detects an arrhythmia. In one embodiment, event detector 213 detects the arrhythmia by detecting heart rate and comparing the heart rate to one or more threshold rates. A bradycardia condition is detected when the heart rate falls below a bradycardia threshold. A tachycardia condition is detected when the heart rate exceeds a tachycardia threshold. In a further embodiment, event detector 213 detects the arrhythmia also by detecting morphological features of the electrogram to one or more predetermined templates. In one specific embodiment, event detector 213 includes an atrial fibrillation detector. In one specific embodiment, event detector 213 includes a ventricular fibrillation detector.

In one embodiment, sensor 212 senses a physiological signal indicative of ischemia, and event detector 213 includes an ischemia detector. In one specific embodiment, sensor 212 senses an electrogram and event detector 213 runs an automatic ischemia detection algorithm to detect an ischemic condition from the electrogram. One specific example of an electrogram-based ischemia detector is

discussed in Zhu et al., U.S. Patent Application No. 09/962,852, entitled "EVOKED RESPONSE SENSING FOR ISCHEMIA DETECTION," filed on September 25, 2001, assigned to Cardiac Pacemakers, Inc., which is incorporated herein by reference in its entirety. In another embodiment, sensor 212 includes an electrical

5   impedance based sensor using a low carrier frequency (e.g., 100 Hz), and event detector 213 runs an automatic ischemia detection algorithm to detect an ischemic condition from the electrical impedance signal. Tissue electrical impedance has been shown to increase significantly during ischemia, as discussed in Min, et al. International Journal of Bioelectromagnetism, 5(1): 53 (2003). Sensor 212 senses

10   low frequency electrical impedance signal between electrodes interposed in the heart. Event detector 213 detects the ischemia as abrupt changes in impedance (such as abrupt increases in value). In another specific embodiment, sensor 212 includes a local heart motion based sensor utilizing an accelerometer located within a lead body positioned on or in the heart, and event detector 213 runs an automatic

15   ischemia detection algorithm to detect an ischemic condition from the acceleration signal. Event detector 213 detects ischemia as an abrupt decrease in the amplitude of local cardiac accelerations.

In one embodiment, sensor 212 includes a metabolic sensor that senses a metabolic signal indicative of a cardiac metabolic level (rate of metabolism of

20   cardiac cells). Examples of the metabolic sensor include a pH sensor, an oxygen pressure (PO_2) sensor, a carbon dioxide pressure (PCO_2) sensor, a glucose sensor, a creatine sensor, a C-creative protein sensor, a creatine kinase sensor, a creatine kinase-MB sensor, and any combination of such sensors. Event detector 213 determines the cardiac metabolic level from the metabolic signal and compares the

25   cardiac metabolic level to one or more predetermined thresholds defining a normal cardiac metabolic range. The abnormal condition is detected when the cardiac metabolic level is outside of the normal cardiac metabolic range.

In one embodiment, sensor 212 includes an impedance sensor to measure pulmonary impedance, or impedance of a portion of the thoracic cavity. Event

30   detector 213 detects the abnormal condition when the impedance is out of its normal range. For example, pulmonary edema, i.e., fluid retention in the lungs resulting

from the decreased cardiac output, increases the pulmonary or thoracic impedance. In one specific embodiment, event detector 213 produces the alert signal when the pulmonary or thoracic impedance exceeds a predetermined threshold impedance. In one embodiment, the impedance sensor is a respiratory sensor that senses the

5 patient's minute ventilation. An example of an impedance sensor sensing minute ventilation is discussed in U.S. Patent No. 6,459,929, "IMPLANTABLE CARDIAC RHYTHM MANAGEMENT DEVICE FOR ASSESSING STATUS OF CHF PATIENTS," assigned to Cardiac Pacemakers, Inc., which is incorporated herein by reference in its entirety.

10 In one embodiment, sensor 212 includes a pressure sensor. Abnormal conditions including arrhythmias and heart failure cause pressures in various portions of the cardiovascular system to deviate from their normal ranges. Event detector 213 detects the abnormal condition when a pressure is outside of its normal range. In one specific embodiment, event detector 213 includes a systolic

15 dysfunction detector to detect an abnormal condition related to pressure during the systolic phase of a cardiac cycle. In another specific embodiment, event detector 213 includes a diastolic dysfunction detector to detect an abnormal condition related to pressure during the diastolic phase of a cardiac cycle. Examples of the pressure sensor include but are not limited to a left atrial (LA) pressure sensor, a left

20 ventricular (LV) pressure sensor, an artery pressure sensor, and a pulmonary artery pressure sensor. Pulmonary edema results in elevated LA and pulmonary arterial pressures. A deteriorated LV results in decreased LV and arterial pressures. In various embodiments, event detector 213 detects an abnormal condition when the LA pressure exceeds a predetermined threshold LA pressure level, when the

25 pulmonary arterial pressure exceeds a predetermined threshold pulmonary arterial pressure level, when the LV pressure falls below a predetermined threshold LV pressure level, and/or when the arterial pressure falls below a predetermined threshold LV pressure level. In other embodiments, event detector 213 derives a parameter from one of these pressures, such as a rate of change of a pressure, and

30 produces a signal when the parameter deviates from its normal range. In one embodiment, the LV pressure sensor senses the LV pressure indirectly, by sensing a

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signal having known or predictable relationships with the LV pressure during all or a portion of the cardiac cycle. Examples of such a signal include but are not limited to an LA pressure and a coronary vein pressure. One specific example of measuring the LV pressure using a coronary vein pressure sensor is discussed in U.S. Patent

5   Application Serial No. 10/038,936, "METHOD AND APPARATUS FOR MEASURING LEFT VENTRICULAR PRESSURE," filed on January 4, 2002, assigned to Cardiac Pacemakers, Inc., which is hereby incorporated by reference in its entirety.

In one embodiment, sensor 212 includes a cardiac output or stroke volume

10   sensor. Examples of stroke volume sensing are discussed in U.S. Patent No. 4,686,987, "BIOMEDICAL METHOD AND APPARATUS FOR CONTROLLING THE ADMINISTRATION OF THERAPY TO A PATIENT IN RESPONSE TO CHANGES IN PHYSIOLOGIC DEMAND," and U.S. Patent No. 5,284,136, "DUAL INDIFFERENT ELECTRODE PACEMAKER," both assigned to Cardiac

15   Pacemakers, Inc., which are incorporated herein by reference in their entirety. Event detector 213 detects the abnormal condition when the stroke volume falls below a predetermined threshold level.

In one embodiment, sensor 212 includes a neural activity sensor to detect activities of the sympathetic nerve and/or the parasympathetic nerve. A significant

20   decrease in cardiac output immediately stimulates sympathetic activities, as the autonomic nervous system attempts to compensate for deteriorated cardiac function. In one specific embodiment, the neural activity sensor includes a neurohormone sensor to sense a hormone level of the sympathetic nerve and/or the parasympathetic nerve. Event detector 213 detects the abnormal condition when the hormone level

25   exceeds a predetermined threshold level. In another specific embodiment, the neural activity sensor includes an action potential recorder to sense the electrical activities in the sympathetic nerve and/or the parasympathetic nerve. Event detector 213 detects the abnormal condition when the frequency of the electrical activities in the sympathetic nerve exceeds a predetermined threshold level. Examples of direct

30   and indirect neural activity sensing are discussed in U.S. Patent No. 5,042,497, "ARRHYTHMIA PREDICTION AND PREVENTION FOR IMPLANTED

DEVICES," assigned to Cardiac Pacemakers, Inc., which is hereby incorporated by reference in its entirety.

In one embodiment, sensor 212 includes a heart rate variability detector. Patients suffering acute decompensated heart failure exhibit abnormally low heart rate variability. An example of detecting the heart rate variability is discussed in U.S. Patent No. 5,603,331, "DATA LOGGING SYSTEM FOR IMPLANTABLE CARDIAC DEVICE," assigned to Cardiac Pacemakers, Inc., which is incorporated herein by reference in their entirety. Event detector 213 detects the abnormal condition when the heart rate variability falls below a predetermined threshold level.

In one embodiment, sensor 212 includes a renal function sensor. Acute decompensated heart failure results in peripheral edema primarily because of fluid retention of the kidneys that follows the reduction in cardiac output. The fluid retention is associated with reduced renal output, decreased glomerular filtration, and formation of angiotensin. Thus, in one specific embodiment, the renal function sensor includes a renal output sensor to sense a signal indicative of the renal output. Event detector 213 detects the abnormal condition when the sensed renal output falls below a predetermined threshold. In another specific embodiment, the renal function sensor includes a filtration rate sensor to sense a signal indicative of the glomerular filtration rate. Event detector 213 detects the abnormal condition when the sensed glomerular filtration rate falls below a predetermined threshold. In yet another specific embodiment, the renal function sensor includes a chemical sensor to sense a signal indicative of angiotensin II levels. Event detector 213 detects the abnormal condition when the sensed angiotensin II levels exceed a predetermined threshold level.

In one embodiment, sensor 212 includes an acoustic sensor being a heart sound sensor and/or a respiratory sound sensor. Arrhythmias and/or heart failure cause abnormal cardiac and pulmonary activity patterns and hence, deviation of heart sounds and respiratory sounds from their normal ranges of pattern and/or amplitude. Event detector 213 detects the abnormal condition when the heart sound or respiratory sound is out of its normal range. For example, detection of the third heart sound (S3) is known to indicate heart failure. In one specific embodiment,

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event detector 213 detects the abnormal condition when the S3 amplitude or amount of S3 activity exceeds a predetermined threshold level. An example of using S3 activity to monitor for heart failure is discussed in U.S. Patent Application Serial No. 10/746,874, "A THIRD HEART SOUND ACTIVITY INDEX FOR HEART

5   FAILURE MONITORING," filed on December 24, 2003, assigned to Cardiac Pacemakers, Inc., which is hereby incorporated by reference in its entirety.

Embodiments of sensor 212 and event detector 213 are discussed in this document by way of example, but not by way of limitation. In various embodiment, sensor 212 and event detector 213 may include combinations of various sensors and

10   detectors discussed above. Other methods and sensors for directly or indirectly detecting an abnormal condition treatable by the gene regulatory therapy are generally useable by gene regulatory system 100.

In one specific embodiment, gene regulatory system 100 is used to treat heart failure. Sensor 212 includes a heart failure sensor sensing a signal indicative of heart failure. Examples of such a heart failure sensor include, but are not limited

15   to, the impedance sensor, the pressure sensor, the cardiac output or stroke volume sensor, the neural activity sensor, the HRV sensor, the renal function sensor, and the acoustic sensor, which are discussed above. These sensors each sense a parameter indicative of heart failure or a symptom associated with heart failure, including

20   acute decompensated heart failure. The heart failure sensor includes one or more of these sensors and any other sensors capable of sensing a signal and producing a parameter indicative of heart failure. In one embodiment, sensor 212 detects acute decompensated heart failure. Examples of detectors detecting acute decompensated heart failure are discussed in U.S. Patent Application Serial No. 10/742,574,

25   "DRUG DELIVERY SYSTEM AND METHOD EMPLOYING EXTERNAL DRUG DELIVERY DEVICE IN CONJUNCTION WITH COMPUTER NETWORK," filed on December 19, 2003, assigned to Cardiac Pacemakers, Inc., which is hereby incorporated by reference in its entirety.

Implantable CRM device 110A includes a hermetically sealed metal can to

30   house at least portion of the electronics of the device. In one embodiment, sensor

212 resides within the metal can. In another embodiment, sensor 212 is outside of the metal can. In one embodiment, sensor 212 is incorporated into lead system 108. In one embodiment, sensor 212 is an external sensor communicating with implantable CRM device 110A. While implantable devices are specifically

5   discussed as an example, the underlying concept can be implemented with either implantable medical devices, external (non-implantable) medical devices, or a combination of both.

FIG. 3 is a block diagram showing another embodiment of the circuit of portions of system 100 including implantable CRM device 110A, lead system 108,

10   implantable gene regulatory signal delivery device 130, and external system 155. Implantable CRM device 110B as shown in FIG. 3 represents one specific embodiment of implantable CRM device 110 and includes pacing and defibrillation capabilities. In addition to controlling the gene regulatory therapy, implantable CRM device 110B delivers therapies including, but not being limited to,

15   bradyarrhythmia pacing, anti-tachyarrhythmia pacing, atrial and/or ventricular cardioversion/defibrillation, CRT, RCT, and drug delivery. However, such therapeutic capabilities are not necessary for system 100 to control the gene regulatory therapy, and hence, are not necessary elements of implantable CRM device 110B. In other words, implantable CRM device 110B can be an implantable

20   pacemaker and/or defibrillator with additional functions including control of the gene regulatory therapy, or it can be a dedicated implantable gene regulatory therapy controller.

In one embodiment, implantable CRM device 110B includes sensor 212, event detector 213, implant controller 214, pacing circuit 320, defibrillation circuit

25   324, and implant telemetry module 316. Pacing circuit 320 delivers pacing pulses to one or more cardiac regions as controlled by implant controller 214. Defibrillation circuit 324 delivers cardioversion or defibrillation shocks to one or more cardiac regions as controlled by implant controller 214. Sensor 212 senses a physiological signal indicative of an abnormal condition treatable by the gene

30   regulatory therapy, and event detector 213 detects that abnormal condition, as discussed above with reference to FIG. 2. In one specific embodiment, in which

implantable CRM device provides for CRT and RCT pacing as well as defibrillation, implant controller 214 includes gene regulation control module 325, a CRT control module 321, an RCT control module 322, a defibrillation control module 323, and a command receiver 326. Gene regulation control module 325

5   generates the gene regulatory control signal in response to an abnormal condition detected by event detector 213 or a gene regulatory command received by command receiver 326. Command receiver 326 receives the gene regulatory command from external system 155 via telemetry link 140. CRT control module 321 controls the delivery of pacing pulses from pacing circuit 320 by executing a CRT algorithm.

10   RCT control module 321 controls the delivery of pacing pulses from pacing circuit 320 by executing a RCT algorithm. Defibrillation control module 323 controls the delivery of cardioversion/defibrillation shocks from defibrillation circuit 324 when a tachyarrhythmic condition is detected. In one embodiment, defibrillation control module 323 includes an atrial defibrillation control module to control the delivery of

15   cardioversion/defibrillation shocks to one or more of the atria. In one embodiment, defibrillation control module 323 includes a ventricular defibrillation control module to control the delivery of cardioversion/defibrillation shocks to one or more of the ventricles.

Implantable CRM device 110B includes a hermetically sealed metal can to

20   house at least portion of the electronics of the device. In one embodiment, sensor 212 resides within the metal can. In another embodiment, sensor 212 is outside of the metal can. In one embodiment, sensor 212 is incorporated into lead system 108. In one embodiment, sensor 212 is an external sensor communicating with implantable CRM device 110B.

25   Lead system 108 includes one or more leads connecting implantable CRM device 110B and implantable gene regulatory signal delivery device 130, referenced as lead system 108A, and pacing leads, defibrillation leads, pacing-defibrillation leads, or any combination of such leads, referenced as lead system 108B. Lead system 108B allows sensing of electrical signals from various regions of heart 101

30   and/or delivery of pacing pulses and/or defibrillation shocks to various regions of heart 101. The various regions of heart 101 includes regions within or about the

right atrium (RA), left atrium (LA), right ventricle (RV), and left ventricle (LV). In one embodiment, lead system 108B includes one or more transvenous leads each having at least one sensing-pacing or defibrillation electrode disposed within heart 101. In one embodiment, lead system 108B includes one or more epicardial leads

5   each having at least one sensing-pacing or defibrillation electrode disposed on heart 101. In one embodiment, lead system 108B includes at least one atrial defibrillation electrode disposed in or about one or both of the atria to allow atrial defibrillation. In one embodiment, lead system 108B includes at least one ventricular defibrillation electrode disposed in or about one or both of the ventricles to allow ventricular

10   defibrillation. In one embodiment, sensor 212 includes at least portions of lead system 108A or 108B. In another embodiment, sensor 212 is incorporated into lead system 108A or 108B.

External system 155 includes external telemetry module 352, external user input device 354, presentation device 356, and external controller 358. These

15   system components distribute in one or more of external device 150, network 160, and remote device 170, depending on design and medical considerations. User input device 354 receives commands and/or parameters from the user and/or the patient to control deliveries of therapy, including the gene regulatory therapy, i.e., the delivery of the one or more gene regulatory signals. Presentation device 356

20   displays or otherwise presents signals acquired and/or abnormal conditions detected by implantable CRM device 110B. External controller 358 controls the operation of external system 155. In one embodiment, external controller 358 further provides automatic control of operations of implantable CRM device 110B. In one embodiment, user input device 352 receives the gene regulatory command entered

25   by the user based on observations of the signals and/or abnormal conditions presented by presentation device 356. In another embodiment, user input device 352 receives the gene regulatory command entered by a patient when the patient physically senses a symptom indicative of an immediate need for the gene regulatory therapy, or entered by a person near the patient who observes a symptom

30   indicative of the immediate need for the gene regulatory therapy. In a further embodiment, external controller 358 automatically analyzes the signals acquired

and/or abnormal conditions detected by implantable CRM device 110B, and generates the gene regulatory command when deemed necessary as a result of the analysis.

Telemetry link 140 is a wireless bidirectional data transmission link
5   supported by implant telemetry module 316 and external telemetry module 352. In one embodiment, telemetry link 140 is an inductive couple formed when two coils – one connected to implant telemetry module 316 and the other connected to external telemetry module 352 – are placed near each other. In another embodiment, telemetry link 140 is a far-field radio-frequency telemetry link allowing implantable
10   CRM device 110B and external system 155 to communicate over a telemetry range that is at least ten feet.

Disorders Amenable to Treatment

The systems and methods of the invention may be used to prevent, inhibit or treat one or more symptoms of any condition amenable to treatment, prophylactic or
15   otherwise, by gene therapy. In one embodiment, the systems of the invention are useful to treat, inhibit or prevent one or more symptoms of a cardiovascular condition. Cardiovascular conditions include but are not limited to coronary artery disease/ischemia, coronary artery disease (CAD), ischemia, angina (chest pain), thrombosis, coronary thrombosis, myocardial infarction (MI), silent ischemia,
20   stenosis/restenosis, transient ischemic attack (TIA), atherosclerosis, peripheral vascular disease, bradyarrhythmia, e.g., bradyarrhythmia, bradycardia, sick sinus rhythm (Sick Sinus Syndrome), sinus bradycardia, sinoatrial block, asystole, sinus arrest, syncope, first degree atrio-ventricular (AV) block, second degree atrio-ventricular (AV) block, third degree atrio-ventricular (AV) block, chronotropic
25   incompetence, tachyarrhythmia, e.g., tachyarrhythmia, tachycardia, fibrillation, flutter, atrial fibrillation, atrial flutter, familial atrial fibrillation, paroxysmal atrial fibrillation, permanent atrial fibrillation, persistent atrial fibrillation, supraventricular tachyarrhythmias, sinus tachycardia, reentry (reentrant arrhythmias), AV nodal reentry, focal arrhythmia, ectopy, ventricular fibrillation
30   (VF), ventricular tachycardia (VT), Wolff-Parkinson-White Syndrome (WPW) and sudden cardiac death, heart failure, e.g., heart failure, cardiomyopathy, congestive

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heart failure, hypertrophic cardiomyopathy, remodeling, non-ischemic cardiomyopathy, dilated cardiomyopathy, restrictive cardiomyopathy, diastolic heart failure, systolic heart failure, and chronic heart failure, heart block/electrical disorders, e.g., atrioventricular (AV) block, bundle branch block (BBB), left bundle

5 branch block (LBBB), right bundle branch block (RBBB), Long QT Syndrome (LQTS), premature ventricular contraction (PVC), electrical remodeling, intraventricular conduction defect, and hemiblock, hemodynamic deficiency, e.g., hypertension, hypotension, left ventricular dysfunction, low ejection fraction, low cardiac output, and low stroke volume, sudden cardiac death, cardiac arrest, sudden

10 cardiac death (SCD), ventricular fibrillation, and pump failure, as well as bacterial endocarditis, viral myocarditis, pericarditis, rheumatic heart disease, and syncope. . In particular, a cardiovascular condition includes, but is not limited to, arrhythmia, e.g., atrial fibrillation, ventricular fibrillation or bradycardia, ischemia, heart failure and hyperplasia not associated with neoplastic disease, which may be associated

15 with ventricular remodeling, diastolic dysfunction, aberrant body temperature, aberrant or altered pressure, e.g., altered venous, left ventricular or left atrial pressure, aberrant or altered heart rate or sounds, aberrant or altered electrogram, aberrant or altered cardiac metabolism, such as altered blood pH, glucose, PO_2, PCO_2, minute ventilation, creatine, CRP, Mef2A, creatine kinase or creatine kinase

20 MB levels, aberrant or altered pulmonary or thoracic impedence, aberrant or altered stroke volume, aberrant or altered neurohormone levels, aberrant or altered electrical activity, aberrant or altered sympathetic nerve activity, aberrant or altered renal output, aberrant or altered filtration rate, aberrant or altered angiotensin II levels, or aberrant or altered respiratory sounds.

25   For instance, cardiovascular conditions associated with certain defective genes (see Table 1), e.g., disease alleles, may be treated by gene therapy.

Table 1

Condition	Current	Defective gene
LQT-1	I_{KS}, amplitude	KVLQTI(KCNQ1)
LQT-2	I_{Kr}, amplitude	HERG (KCNH2)
LQT-3	I_{Na}, late current	SCN5a ($hNaV_{1.5}$)
LQT-5	I_{KS}, amplitude	MinK (KCNE1)

LQT-6	I_{Kr}, deactivation	MiRPI (KCNE2)
LQT-7	I_{KI}, amplitude	Kir2.1(KCNj2)
JLN-1	I_{KS}, amplitude	KVLQTI(KCNQl)
JLN-2	I_{Kr}, amplitude	MinK(KCNEI)
SIDS-1	I_{Na}, late current	SCN5a(hNaV$_{1.5}$)
SIDS-2	I_{KS}, amplitude	KVLQTI(KCNQ1)
Brugada syndrome	I_{Na}, amplitude	SCN5a(hNaV$_{1.5}$)
IVF	I_{Na}, amplitude	SCN5a(hNaV$_{1.5}$)
CVT	Cell Ca^{2+}	RyR receptor
CCD	I_{Na}, amplitude	SCN5a(hNaV$_{1.5}$)

JLN = Jerrell & Lange-Nielsen; SIDS = sudden infant death syndrome; IVF = idiopathic ventricular fibrillation; CVT = catecholaminergic ventricular tachycardia; CCD = cardiac conduction disease

Gene Therapy Vectors

Gene therapy vectors include, for example, viral vectors, liposomes and other lipid-containing complexes, and other macromolecular complexes capable of mediating delivery of a gene to a host cell. Vectors can also comprise other components or functionalities that further modulate gene delivery and/or gene expression, or that otherwise provide beneficial properties to the targeted cells. Such other components include, for example, components that influence binding or targeting to cells (including components that mediate cell-type or tissue-specific binding); components that influence uptake of the vector by the cell; components that influence localization of the transferred gene within the cell after uptake (such as agents mediating nuclear localization); and components that influence expression of the gene. Such components also might include markers, such as detectable and/or selectable markers that can be used to detect or select for cells that have taken up and are expressing the nucleic acid delivered by the vector. Such components can be provided as a natural feature of the vector (such as the use of certain viral vectors which have components or functionalities mediating binding and uptake), or vectors can be modified to provide such functionalities. Selectable markers can be positive, negative or bifunctional. Positive selectable markers allow selection for cells carrying the marker, whereas negative selectable markers allow cells carrying the marker to be selectively eliminated. A variety of such marker genes have been described, including bifunctional (i.e., positive/negative) markers

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(see, e.g., WO 92/08796; and WO 94/28143). Such marker genes can provide an added measure of control that can be advantageous in gene therapy contexts. A large variety of such vectors are known in the art and are generally available.

Gene therapy vectors within the scope of the invention include, but are not limited to, isolated nucleic acid, e.g., plasmid-based vectors which may be extrachromosomally maintained, and viral vectors, e.g., recombinant adenovirus, retrovirus, lentivirus, herpesvirus, poxvirus, papilloma virus, or adeno-associated virus, including viral and non-viral vectors which are present in liposomes, e.g., neutral or cationic liposomes, such as DOSPA/DOPE, DOGS/DOPE or DMRIE/DOPE liposomes, and/or associated with other molecules such as DNA-anti-DNA antibody-cationic lipid (DOTMA/DOPE) complexes. Exemplary gene therapy vectors are described below. Gene therapy vectors may be administered via any route including, but not limited to, intramuscular, buccal, rectal, intravenous or intracoronary administration, and transfer to cells may be enhanced using electroporation and/or iontophoresis.

Retroviral vectors

Retroviral vectors exhibit several distinctive features including their ability to stably and precisely integrate into the host genome providing long-term transgene expression. These vectors can be manipulated *ex vivo* to eliminate infectious gene particles to minimize the risk of systemic infection and patient-to-patient transmission. Pseudotyped retroviral vectors can alter host cell tropism.

Lentiviruses

Lentiviruses are derived from a family of retroviruses that include human immunodeficiency virus and feline immunodeficiency virus. However, unlike retroviruses that only infect dividing cells, lentiviruses can infect both dividing and nondividing cells. For instance, lentiviral vectors based on human immunodeficiency virus genome are capable of efficient transduction of cardiac myocytes *in vivo*. Although lentiviruses have specific tropisms, pseudotyping the viral envelope with vesicular stomatitis virus yields virus with a broader range (Schnepp et al., Meth. Mol. Med., 69:427 (2002)).

Adenoviral vectors

Adenoviral vectors may be rendered replication-incompetent by deleting the early (E1A and E1B) genes responsible for viral gene expression from the genome and are stably maintained into the host cells in an extrachromosomal form. These vectors have the ability to transfect both replicating and nonreplicating cells and, in particular, these vectors have been shown to efficiently infect cardiac myocytes *in vivo*, e.g., after direction injection or perfusion. Adenoviral vectors have been shown to result in transient expression of therapeutic genes *in vivo*, peaking at 7 days and lasting approximately 4 weeks. The duration of transgene expression may be improved in systems utilizing cardiac specific promoters. In addition, adenoviral vectors can be produced at very high titers, allowing efficient gene transfer with small volumes of virus.

Adeno-associated virus vectors

Recombinant adeno-associated viruses (rAAV) are derived from nonpathogenic parvoviruses, evoke essentially no cellular immune response, and produce transgene expression lasting months in most systems. Moreover, like adenovirus, adeno-associated virus vectors also have the capability to infect replicating and nonreplicating cells and are believed to be nonpathogenic to humans. Moreover, they appear promising for sustained cardiac gene transfer (Hoshijima et al,. Nat. Med., 8:864 (2002); Lynch et al., Circ. Res., 80:197 (1997)).

Herpesvirus/amplicon

Herpes simplex virus 1 (HSV-1) has a number of important characteristics that make it an important gene delivery vector *in vivo*. There are two types of HSV-1-based vectors: 1) those produced by inserting the exogenous genes into a backbone virus genome, and 2) HSV amplicon virions that are produced by inserting the exogenous gene into an amplicon plasmid that is subsequently replicated and then packaged into virion particles. HSV-1 can infect a wide variety of cells, both dividing and nondividing, but has obviously strong tropism towards nerve cells. It has a very large genome size and can accommodate very large transgenes (>35 kb). Herpesvirus vectors are particulary useful for delivery of large genes, e.g., genes encoding ryanodine receptors and titin.

Plasmid DNA vectors

Plasmid DNA is often referred to as "naked DNA" to indicate the absence of a more elaborate packaging system. Direct injection of plasmid DNA to myocardial cells *in vivo* has been accomplished. Plasmid-based vectors are relatively
5   nonimmunogenic and nonpathogenic, with the potential to stably integrate in the cellular genome, resulting in long-term gene expression in postmitotic cells *in vivo*. For example, expression of secreted angiogenesis factors after muscle injection of plasmid DNA, despite relatively low levels of focal transgene expression, has demonstrated significant biologic effects in animal models and appears promising
10   clinically (Isner, Nature, 415:234 (2002)). Furthermore, plasmid DNA is rapidly degraded in the blood stream; therefore, the chance of transgene expression in distant organ systems is negligible. Plasmid DNA may be delivered to cells as part of a macromolecular complex, e.g., a liposome or DNA-protein complex, and delivery may be enhanced using techniques including electroporation.

15   Synthetic oligonucleotides

Antisense oligonucleotides are short (approximately 10 to 30 nucleotides in length), chemically synthesized DNA molecules that are designed to be complementary to the coding sequence of an RNA of interest. These agents may enter cells by diffusion or liposome-mediated transfer and possess relatively high
20   transduction efficiency. These agents are useful to reduce or ablate the expression of a targeted gene while unmodified oligonucleotides have a short half-life *in vivo*, modified bases, sugars or phosphate groups can increase the half-life of oligonucleotide. For unmodified nucleotides, the efficacy of using such sequences is increased by linking the antisense segment with a specific promoter of interest,
25   e.g., in an adenoviral construct. In one embodiment, electroporation and/or liposomes are employed to deliver plasmid vectors. Synthetic oligonucleotides may be delivered to cells as part of a macromolecular complex, e.g., a liposome, and delivery may be enhanced using techniques such as electroporation.

Regulatable Transcriptional Control Elements

30   The device of the invention may deliver one or more signals including, but not limited to, light of a particular wavelength or a range of wavelengths, light of a

particular energy, acoustic energy, an electric field, a chemical, electromagnetic energy, thermal energy or other forms of temperature or matter, which signal is recognized by a regulatable transcriptional control element in a gene therapy vector.

A variety of strategies have been devised to control *in vivo* expression of
5   transferred genes and thus alter the pharmacokinetics of *in vivo* gene transfer vectors in the context of regulatable or inducible promoters. Many of these regulatable promoters use exogenously administered agents to control transgene expression and some use the physiologic milieu to control gene expression. Examples of the exogenous control promoters include the tetracycline-responsive promoter, a
10   chimeric transactivator consisting of the DNA and tetracycline-binding domains from the bacterial *tet* repressor fused to the transactivation domain of herpes simplex virion protein 16 (Ho et al., Brain Res. Mol. Brain Res., 41:200 (1996)); a chimeric promoter with multiple cyclic adenosine monophosphate response elements superimposed on a minimal fragment of the 5'-flanking region of the cystic
15   fibrosis transmembrane conductance regulator gene (Suzuki et al., 7:1883 (1996)); the EGR1 radiation-inducible promoter (Hallahan et al., Nat. Med., 1:786 (1995)); and the chimeric GRE promoter (Lee et al., J. Thoracic Cardio. Surg., 118:26 (1996)), with 5 GREs from the rat tyrosine aminotransferase gene in tandem with the insertion of Ad2 major late promoter *TATA* box-initiation site (Narumi et al.,
20   Blood, 92:812 (1998)). Examples of the physiologic control of promoters include a chimera of the thymidine kinase promoter and the thyroid hormone and retinoic acid-responsive element responsive to both exogenous and endogenous tri-iodothyroniine (Hayashi et al., J. Biol. Chem., 269:23872 (1994)); complement factor 3 and serum amyloid A3 promoters responsive to inflammatory stimuli; the
25   grp78 and BiP stress-inducible promoter, a glucose-regulated protein that is inducible through glucose deprivation, chronic anoxia, and acidic pH (Gazit et al., Cancer Res., 55:1660 (1995)); and hypoxia-inducible factor 1 and a heterodimeric basic helix-loop-helix protein that activates transcription of the human erythropoietin gene in hypoxic cells, which has been shown to act as a regulatable
30   promoter in the context of gene therapy *in vivo* (Forsythe et al., Mol. Cell Biol., 16:4604 (1996)).

Regulatable transcriptional elements useful in gene therapy vectors and methods of the invention include, but are not limited to, a truncated ligand binding domain of a progesterin receptor (controlled by antiprogestin), a *tet* promoter (controlled by *tet* and dox) (Dhawan et al., Somat. Cell. Mol. Genet., 21, 233

5   (1995); Gossen et al., Science, 268:1766 (1995); Gossen et al., Science, 89:5547 (1992); Shockett et al., Proc. Natl. Acad. Sci. USA, 92, 6522 (1995)), hypoxia-inducible nuclear factors (Semenza et al., Proc. Natl. Acad. Sci. USA, 88, 5680 (1991); Semenza et al., J. Biol. Chem., 269, 23757)), steroid-inducible elements and promoters, such as the glucocorticoid response element (GRE) (Mader and White,

10   Proc. Natl. Acad. Sci. USA, 90, 5603 (1993)), and the fusion consensus element for RU486 induction (Wang et al., Proc. Natl. Acad. Sci. USA, 91:818 (1994)), those sensitive to electromagnetic fields, e.g., those present in metallothionein I or II, c-myc, and HSP70 promoters (Lin et al., J. Cell. Biochem., 81:143 (2001); Lin et al., J. Cell. Biochem., 54:281 (1994); U.S. published application 20020099026)), and

15   electric pulses (Rubenstrunk et al., J. Gene Med., 5:773 (2003)), as well as a yeast GAL4/TATA promoter, auxin inducible element, an ecdysone responsive element (No et al., Proc. Natl. Acad. Sci. USA, 93:3346 (1996)), an element inducible by rapamycin (FK506) or an analog thereof (Rivera et al., Nat. Med., 2:1028 (1996); Ye et al., Science, 283:88 (1999); Rivera et al., Proc. Natl. Acad. Sci. USA, 96:8657

20   (1999)), a tat responsive element, a metal, e.g., zinc, inducible element, a radiation inducible element, e.g., ionizing radiation has been used as the inducer of the promoter of the early growth response gene (Erg-1) (Hallahan et al., Nat. Med., 1:786 (1995)), an element which binds nuclear receptor PPARγ (peroxisome proliferators activated receptors), which is composed of a minimal promoter fused

25   to PPRE (PPAR responsive elements, see WO 00/78986), a cytochrome P450/A1 promoter, a MDR-1 promoter, a promoter induced by specific cytokines (Varley et al., Nat. Biotech., 15:1002 (1997)), a light inducible element (Shimizu-Sato et al., Nat. Biotech., 20:1041 (2002)), a *lacZ* promoter, and a yeast *Leu*3 promoter.

In some embodiments, cell- or tissue-specific control elements, such as

30   muscle-specific and inducible promoters, enhancers and the like, will be of particular use, e.g., in conjunction with regulatable transcriptional control elements.

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Such control elements include, but are not limited to, those derived from the actin and myosin gene families, such as from the myoD gene family (Weintraub et al., Science, 251, 761 (1991)); the myocyte-specific enhancer binding factor MEF-2 (Cserjesi and Olson, Mol. Cell Biol., 11, 4854 (1991)); control elements derived

5   from the human skeletal actin gene (Muscat et al., Mol. Cell Bio., 7, 4089 (1987)) and the cardiac actin gene; muscle creatine kinase sequence elements (Johnson et al., Mol. Cell Biol., 9, 3393 (1989)) and the murine creatine kinase enhancer (mCK) element; control elements derived from the skeletal fast-twitch troponin C gene, the slow-twitch cardiac troponin C gene and the slow-twitch troponin I genes.

10   Cardiac cell restricted promoters include but are not limited to promoters from the following genes: a α-myosin heavy chain gene, e.g., a ventricular α-myosin heavy chain gene, β-myosin heavy chain gene, e.g., a ventricular β-myosin heavy chain gene, myosin light chain 2v gene, e.g., a ventricular myosin light chain 2 gene, myosin light chain 2a gene, e.g., a ventricular myosin light chain 2 gene,

15   cardiomyocyte-restricted cardiac ankyrin repeat protein (CARP) gene, cardiac α-actin gene, cardiac m2 muscarinic acetylcholine gene, ANP gene, BNP gene, cardiac troponin C gene, cardiac troponin I gene, cardiac troponin T gene, cardiac sarcoplasmic reticulum Ca-ATPase gene, skeletal α-actin gene, as well as an artificial cardiac cell-specific promoter.

20   Further, chamber-specific promoters or enhancers may also be employed, e.g., for atrial-specific expression, the quail slow myosin chain type 3 (MyHC3) or ANP promoter, or the cGATA-6 enhancer, may be employed. For ventricle-specific expression, the iroquois homeobox gene may be employed. Examples of ventricular myocyte-specific promoters include a ventricular myosin light chain 2 promoter and

25   a ventricular myosin heavy chain promoter.

        In other embodiments, disease-specific control elements may be employed. Thus, control elements from genes associated with a particular disease, including but not limited to any of the genes disclosed herein may be employed in vectors of the invention.

30   Nevertheless, other promoters and/or enhancers which are not specific for cardiac cells or muscle cells, e.g., RSV promoter, may be employed in the

expression cassettes and methods of the invention. Other sources for promoters and/or enhancers are promoters and enhancers from the Csx/NKX 2.5 gene, titin gene, α-actinin gene, myomesin gene, M protein gene, cardiac troponin T gene, RyR2 gene, Cx40 gene, and Cx43 gene, as well as genes which bind Mef2,

5   dHAND, GATA, CarG, E-box, Csx/NKX 2.5, or TGF-beta, or a combination thereof.

The response of the regulatable transcriptional control element to one or more intermittent signals, a prolonged signal or different levels of a signal, may be tested *in vitro* or *in vivo*. The vector may include the regulatable transcriptional

10   control element linked to a marker gene, i.e., one which is readily detectable or capable of detection such as green fluorescent protein (GFP). For example, a vector having a promoter which is sensitive to electrical pulses, a MT-I or MT-II promoter (Rubenstruck et al., J. Gene Med., 5:773 (2003)), is linked to an open reading frame for a marker gene. The resulting expression cassette, e.g., one which is introduced

15   to an adenovirus vector or to a plasmid vector, is employed to infect or transfect murine cells, e.g., murine cardiac cells, or heart sections. An electrode system designed for use in a small flask is used to deliver electrical pulses. Then fluorescence in the cells or a lysate thereof is detected, and/or or vector specific RNA is measured, for instance, using RT-PCR, and optionally compared to data

20   from control cells. Similarly, a vector having a promoter which is sensitive to electrical pulses is linked to an open reading frame for a therapeutic gene, e.g., Serca2, introduced to cells, e.g., cardiac cells such as those with decreased levels of the gene product encoded by the therapeutic gene, and the phenotype of the recombinant cells compared to control cells. Vectors may also be introduced to a

25   non-human large animal model, e.g., pigs, to determine the level and spatial expression of the exogenously introduced gene in response to signals, e.g., electrical pulses, from an implantable device in that animal.

Exemplary Genes for Gene Therapy Vectors

Open reading frames useful in gene therapy vectors include those for

30   hepatocyte growth factor, ARK_{Ct}, endothelial GF121, angiotensin type II receptor, p16INK4a, heat shock protein (HSP), e.g., HSP70, sodium channel protein, e.g.,

SCN5A, C reactive protein, connexin, e.g., connexin 40, 41, 43 or 45, sarcoplasmic reticulum Ca2$^+$ ATPase (SERCA2a), ryanodine receptor, MiRPI, cardiac endothelin-1, KCNEI (I_{Ks}), protein kinase C, HIF-1α, p38MAPK, Cox-2, phospholamban, matrix metalloproteinases, adrenergic receptors (AR) and kinases

5   therefore, e.g., betaAR and betaARK, adenylylcyclase, cytochrome oxidase B subunit III, ATP synthase subunit 6, calcium channel proteins such as voltage gated Ca^{2+} channels, potassium channel proteins such as KCNA5(Kv1.5), KCND2(Kv4.2), KCND3 (Kv 4.3, I_{to}), KCNEI (minK), KCNE2, KCNQ1, as well as K+ inwardly rectifying channel such as Kir3.1 (KCNJ3), KCNH2 (HERG, I_{kr}),

10   Kv4.3, Kir3.4, Kir6.1 and Kir6.2, and open reading frames for the sodium-calcium exchanger ($I_{Na/Ca}$), e.g., NCKX1-4. Examples of preferred βAR include β_1-adrenergic receptors or β_2-adrenergic receptors, and preferred adenylylcyclases include a cardiac AC such as AC$_V$ or AC$_{VI}$, more preferably AC$_{VI}$. See also Tables 1 and 2 for genes useful in cardiovascular applications.

Table 2

Subunit	Corresponding Current	Primary Function
HCN	I_f (pacemaking)	Diastolic depolarization
Kir 2.1	I_{k1}	Resting potential, terminal repolarization
Kir3.1/3.4	I_{KACh}	Mediates acetylcholine effects
ERG	I_{Kr} (α-subunit)	Phase-3 repolarization
MiRP1	Modulates I_{Kr}, I_f, I_{to}	
KvLQT1	I_{Ks} (α-subunit)	Phase-3 repolarization (esp. with β–adrenergic stimulation, I_{Kr} inhibition)
mink	I_{Ks} (β-subunit)	Necessary to form I_{Ks} with KvLQT1.
Kv4.2/4.3	I_{to} (α-subunit)	Early (phase-1) repolarization
Kv1.4	I_{to} (α-subunit)	Early (phase-1) repolarization
KChIP2	I_{to}(β-subunit)	Necessary to form I_{to}
Kv1.5/3.1	I_{Kur}	Phase 1-2 repolarization
$Ca_v1.2$	I_{CaL} (α-subunit)	Maintenance of plateau. Electromechanical coupling. Automaticity, conduction SAN, AVN
$Ca_v1.3$	I_{CaL} component	Role in SAN function in mice.
$Ca_v3,103,3$	I_{CaT}	Role in pacemaking
$Na_v1.5$	I_{Na}	Conduction A,V, PF
Cx40,43,45	I_{GJ}	Intercellular conduction

Other genes of interest encode angiogens, acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF), fibroblast growth factor 3 (FGF-3),

5   fibroblast growth factor 4 (FGF-4), fibroblast growth factor 5 (FGF-5), fibroblast growth factor 6 (FGF-6), fibroblast growth factor 7 (FGF-7), fibroblast growth factor 8 (FGF-8), fibroblast growth factor 9 (FGF-9), angiogenin 1, angiogenin 2, platelet-derived endothelial-cell growth factor (PD-ECGF), transforming growth factor-α (TGF-α), transforming growth factor-β (TGF-β), tumor necrosis factor-

10   α (TNF-α), vascular endothelial growth factor 121 (VEGF 121),vascular endothelial growth factor 165 (VEGF 165), vascular endothelial growth factor 189 (VEGF 189), vascular endothelial growth factor 206 (VEGF 206), vascular endothelial growth factor B (VEGF-B), vascular endothelial growth factor C (VEGF-C), vascular endothelial growth factor D (VEGF-D), vascular endothelial growth factor

15   E (VEGF-E), vascular endothelial growth factor F (VEGF-F), angiopoietin-1, angiopoietin-2, thrombospondin (TSP), proliferin, ephrin-A1 (B61), e-selectin,

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chicken chemotactic and angiogenic factor (cCAF), leptin, heparin affin regulatory peptide (HARP), platelet derived growth factor (PDGF), e.g., PDGF-AA, PDGF-AB or PDGF-BB, or heparin.

Thus, in one embodiment, the transgene encodes a gene product including
5 but not limited to an angiogenic protein, e.g., a fibroblast growth factor (FGF) such as acidic-FGF, basic-FGF, and FGF-5, vascular endothelial growth factor (VEGF), e.g., $VEGF_{145}$, $VEGF_{121}$, $VEGF_{120}$, $VEGF_{164}$, $VEGF_{165}$, $VEGF_{189}$, and $VEGF_{206}$, IGF-1, TGF-beta, e.g., $TGF-beta_1$, leukemia inhibitory factor (LIF) alone or in combination with other cytokines, a myogenic factor, e.g., myoD, RyR (cardiac
10 ryanodine receptor), Del I, myogenin, parvalbumin, Myf5, and MRF, transcription factors (GATA such as GATA-4 and dHAND/eHAND), cytokines such as cardiotrophin-1, calsequestrin, neuregulin, for instance, neuregulin 1, 2 or 3, and homeobox gene products, e.g., Csx, tinman, and the NKx family, e.g., NKx 2.5, transferrin, platelet-derived growth factor (PDGF), epidermal growth factor (EGF),
15 adrenocorticotrophin, macrophage colony-stimulating factor, protein kinase C activators, endothelial growth factor, β2 adrenergic receptor (1 or 2), mutant G protein receptor kinase (GRK), adenylyl cylase (AC), e.g., cardiac AC such as human type II, V or VI adenyl cylase (U.S. Patent No. 6,436,672), V2 vasopressin receptor, phospholambam, β-adrenergic receptor kinase, N-cadherin, connexin-40,
20 connexin-42, connexin-43, contractable proteins, e.g., myosin heavy chain (MyHC), myosin light chain (MyLC), myosin binding protein C, actin, tropomyosin, troponin, e.g., troponin T, M protein, tropomodulin, myofibrillar protein, stress related protein, e.g., HSP such as HSP70i, HSP27, HSP40 or HSP60, α-1 antitrypsin, HF1-a, HF-1b, MEF2, BMP-2, BMP-4, BMP-17, BMP-18, Pax7,
25 oxytocin, oxytocin receptor, myocyte nuclear factor, Frzb (see published US application 20020147329), Rb-interacting zinc finger protein (U.S. Patent No. 6,468,985), nNOS, eNOS, iNOS, serine/threonine protein phosphatase, cardiac hypertrophy factor, CT-1, α, β, γ or δ sarcoglycan, hypoxia inducible factor 1α, bcl-2, FasL, cytokine gp 130 receptor, gp130, Akt, adenosine A3 receptor, angiogenin,
30 e.g., angiogenin-1 or angiogenin-2, TNFα, dystrophin, tafazzin, desmin, lamin, troponin C, caspase inhibitors, ERK-type of MAP kinases (p42 and p44, anti-

apoptosis), IL-1B, serum releasing factor, and ILGF (I and II), NGF, growth hormone, e.g., human growth hormone, or angiotensin, e.g., angiotensin II.

In another embodiment, e.g., for cells from a mammal with an inherited or acquired disorder such as one characterized by overexpression of certain

5   endogenous genes (wild type or mutant), the transgene may comprise antisense or ribozyme sequences which substantially correspond to the reverse complement of at least a portion of the endogenous gene, and which antisense or ribozyme sequence, when expressed in a cell of a mammal with the disorder, results in a decrease in the expression of the endogenous gene. Alternatively, the transgene may comprise

10   sequences which, after homologous recombination with the endogenous gene, e.g., a disease allele, result in a decrease in the expression of the endogenous gene and increased expression of the transgene.

Vector or Recombinant Cell Delivery

Several techniques have been developed for cardiac gene delivery, including

15   pericardial infusion, endomyocarial injection, intracoronary injection, coronary venous retroperfusion, and aortic root injection (Isner, Nature, 415:234 (2002)). The different techniques achieve variable response in homogeneity of gene delivery, resulting in focal gene expression within the heart (Hajjar et al., Circ. Res., 86:616 (2000). For this reason, techniques that achieve diffuse uptake would seem to be

20   superior. Two such methods utilize the heart's arterial and venous circulation to accomplish disseminated viral transfection. Arterial injection, performed directly through a percutaneous approach or indirectly by an infusion into the cross-clamped aorta, has shown promise in animal models of heart failure and is appealing in that it can be performed either at the time of cardiac surgery or as percutaneous

25   intervention (Hajjar et al., PNAS USA, 95:5251 (1998)). Similarly, retroperfusion through the coronary sinus appears to produce a more global gene expression in comparison with techniques of localized or focal injection (Boeckstegers et al., Circ., 100:1 (1999)).

Recombinant cells may be administered intravenously, transvenously,

30   intramyocardially or by any other convenient route, and delivered by a needle,

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catheter, e.g., a catheter which includes an injection needle or infusion port, or other suitable device.

Direct myocardial injection

Direct myocardial injection of plasmid DNA as well as virus vectors, e.g.,
5   adenoviral vectors, and cells including recombinant cells has been documented in a number of *in vivo* studies. This technique when employed with plasmid DNA or adenoviral vectors has been shown to result in effective transduction of cardiac myocytes. Thus, direct injection may be employed as an adjunct therapy in patients undergoing open-heart surgery or as a stand-alone procedure via a modified
10 thorascope through a small incision. In one embodiment, this mode of administration is used to deliver a gene or gene product that would only require limited transfection efficiency to produce a significant therapeutic response, such as a gene that encodes for or leads to a secreted product (e.g., VEGF, endothelial nitric oxide synthase). Virus, e.g., pseudotyped, or DNA- or virus-liposome complexes
15 may be delivered intramyocardially.

Catheter-based delivery

Intracoronary delivery of genetic material can result in transduction of approximately 30% of the myocytes predominantly in the distribution of the coronary artery. Parameters influencing the delivery of vectors via intracoronary
20 perfusion and enhancing the proportion of myocardium transduced include a high coronary flow rate, longer exposure time, vector concentration, and temperature. Gene delivery to a substantially greater percent of the myocardium may be enhanced by administering the gene in a low-calcium, high-serotonin mixture (Donahue et al., Nat. Med., 6:1395 (2000)). The potential use of this approach for
25 gene therapy for heart failure may be increased by the use of specific proteins that enhance myocardial uptake of vectors (e.g., cardiac troponin T).

Improved methods of catheter-based gene delivery have been able to achieve almost complete transfection of the myocardium *in vivo*. Hajjar et al. (Proc. Natl. Acad. Sci. USA, 95:5251 (1998)) used a technique combining surgical catheter
30 insertion through the left ventricular apex and across the aortic valve with perfusion of the gene of interest during cross-clamping of the aorta and pulmonary artery.

This technique resulted in almost complete transduction of the heart and could serve as a protocol for the delivery of adjunctive gene therapy during open-heart surgery when the aorta can be cross-clamped.

Recombinant cells may also be delivered via catheter.

5   Pericardial delivery

Gene delivery to the ventricular myocardium by injection of genetic material into the pericardium has shown efficient gene delivery to the epicardial layers of the myocardium. However, hyaluronidase and collagenase may enhance transduction without any detrimental effects on ventricular function. Recombinant cells may also

10   be delivered pericardially.

Intravenous delivery

Intravenous gene delivery may be efficacious for myocardial gene delivery. However, to improve targeted delivery and transduction efficiency of intravenously administered vectors, targeted vectors may be employed. In one embodiment,

15   intravenous administration of DNA-liposome or antibody-DNA complexes may be employed.

Lead-based delivery

Gene delivery can be performed by incorporating a gene delivery device or lumen into a lead such as a pacing lead, defibrillation lead, or pacing-defibrillation

20   lead. An endocardial lead including a gene delivery device or lumen allows gene delivery to the endocardial layers of the myocardium. An epicardial lead including a gene delivery device or lumen allows gene delivery to the endocardial layers of the myocardium. A transvenous lead including a gene delivery device or lumen may also allow intravenous gene delivery. Lead-based delivery is particularly

25   advantageous when the lead is used to deliver electrical and gene therapies to the same region.

Generally any route of administration may be employed, including oral, mucosal, intramuscular, buccal and rectal administration. Fro certain vectors, certain route of administration may be preferred. For instance, viruses, e.g.,

30   pseudotypsed virus, and DNA- or virus-liposome, e.g., HVJ-liposome, may be

administered by coronary infusion, while HVJ-liposome complexes may be delivered pericardially.

Recombinant cells may also be delivered systemically, e.g., intravenously.

Targeted Vectors

5   The present invention contemplates the use of cell targeting not only by delivery of the transgene or recombinant cell into the coronary artery, for example, but also by use of targeted vector constructs having features that tend to target gene delivery and/or gene expression to particular host cells or host cell types (such as the myocardium). Such targeted vector constructs would thus include targeted delivery

10   vectors and/or targeted vectors, as described herein. Restricting delivery and/or expression can be beneficial as a means of further focusing the potential effects of gene therapy. The potential usefulness of further restricting delivery/expression depends in large part on the type of vector being used and the method and place of introduction of such vector. For instance, delivery of viral vectors via intracoronary

15   injection to the myocardium has been observed to provide, in itself, highly targeted gene delivery. In addition, using vectors that do not result in transgene integration into a replicon of the host cell (such as adenovirus and numerous other vectors), cardiac myocytes are expected to exhibit relatively long transgene expression since the cells do not undergo rapid turnover. In contrast, expression in more rapidly

20   dividing cells would tend to be decreased by cell division and turnover. However, other means of limiting delivery and/or expression can also be employed, in addition to or in place of the illustrated delivery method, as described herein.

Targeted delivery vectors include, for example, vectors (such as viruses, non-viral protein-based vectors and lipid-based vectors) having surface components

25   (such as a member of a ligand-receptor pair, the other half of which is found on a host cell to be targeted) or other features that mediate preferential binding and/or gene delivery to particular host cells or host cell types. As is known in the art, a number of vectors of both viral and non-viral origin have inherent properties facilitating such preferential binding and/or have been modified to effect

30   preferential targeting (see, e.g., Miller, et al., FASEB Journal, 9:190 (1995); Chonn et al., Curr. Opin. Biotech., 6:698 (1995); Schofield et al., British Med. Bull., 51:56

(1995); Schreier, Pharmaceutica Acta Helvetiae, 68:145 (1994); Ledley, Human Gene Therapy, 6:1129 (1995); WO 95/34647; WO 95/28494; and WO 96/00295).

Targeted vectors include vectors (such as viruses, non-viral protein-based vectors and lipid-based vectors) in which delivery results in transgene expression
5 that is relatively limited to particular host cells or host cell types. For example, transgenes can be operably linked to heterologous tissue-specific enhancers or promoters thereby restricting expression to cells in that particular tissue. For example, tissue-specific transcriptional control sequences derived from a gene encoding left ventricular myosin light chain-2 (MLC_2V) or myosin heavy chain
10 (MHC) can be fused to a transgene within a vector. Expression of the transgene can therefore be relatively restricted to ventricular cardiac myocytes.

Dosages and Dosage Forms

The amount of gene therapy vector(s), e.g., those which are present in a recombinant cell or in acellular form, administered and device based signal emitted
15 to achieve a particular outcome will vary depending on various factors including, but not limited to, the gene and promoter chosen, the condition, patient specific parameters, e.g., height, weight and age, and whether prevention or treatment is to be achieved. The gene therapy vector/device system of the invention is amenable to chronic use for prophylactic purposes.

20 Vectors of the invention may conveniently be provided in the form of formulations suitable for administration, e.g., into the blood stream (e.g., in an intracoronary artery). A suitable administration format may best be determined by a medical practitioner for each patient individually, according to standard procedures. Suitable pharmaceutically acceptable carriers and their formulation are described in
25 standard formulations treatises, e.g., Remington's Pharmaceuticals Sciences. Vectors of the present invention should preferably be formulated in solution at neutral pH, for example, about pH 6.5 to about pH 8.5, more preferably from about pH 7 to 8, with an excipient to bring the solution to about isotonicity, for example, 4.5% mannitol or 0.9% sodium chloride, pH buffered with art-known buffer
30 solutions, such as sodium phosphate, that are generally regarded as safe, together with an accepted preservative such as metacresol 0.1% to 0.75%, more preferably

from 0.15% to 0.4% metacresol. Obtaining a desired isotonicity can be accomplished using sodium chloride or other pharmaceutically acceptable agents such as dextrose, boric acid, sodium tartrate, propylene glycol, polyols (such as mannitol and sorbitol), or other inorganic or organic solutes. Sodium chloride is

5   preferred particularly for buffers containing sodium ions. If desired, solutions of the above compositions can also be prepared to enhance shelf life and stability. Therapeutically useful compositions of the invention can be prepared by mixing the ingredients following generally accepted procedures. For example, the selected components can be mixed to produce a concentrated mixture which may then be

10   adjusted to the final concentration and viscosity by the addition of water and/or a buffer to control pH or an additional solute to control tonicity.

The vectors can be provided in a dosage form containing an amount of a vector effective in one or multiple doses. For viral vectors, the effective dose may be in the range of at least about 10^7 viral particles, preferably about 10^9 viral

15   particles, and more preferably about 10^{11} viral particles. The number of viral particles may, but preferably does not exceed 10^{14}. As noted, the exact dose to be administered is determined by the attending clinician, but is preferably in 1 ml phosphate buffered saline. For delivery of recombinant cells, the number of cells to be administered will be an amount which results in a beneficial effect to the

20   recipient. For example, from 10^2 to 10^{10}, e.g., from 10^3 to 10^9, 10^4 to 10^8, or 10^5 to 10^7, cells can be administered. For delivery of plasmid DNA alone, or plasmid DNA in a complex with other macromolecules, the amount of DNA to be administered will be an amount which results in a beneficial effect to the recipient. For example, from 0.0001 to 1 mg or more, e.g., up to 1 g, in individual or divided

25   doses, e.g., from 0.001 to 0.5 mg, or 0.01 to 0.1 mg, of DNA can be administered.

In one embodiment, in the case of heart disease, administration may be by intracoronary injection to one or both coronary arteries (or to one or more saphenous vein or internal mammary artery grafts or other conduits) using an appropriate coronary catheter. A variety of catheters and delivery routes can be used

30   to achieve intracoronary delivery, as is known in the art. For example, a variety of general purpose catheters, as well as modified catheters, suitable for use in the

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present invention are available from commercial suppliers. Also, where delivery to the myocardium is achieved by injection directly into a coronary artery, a number of approaches can be used to introduce a catheter into the coronary artery, as is known in the art. By way of illustration, a catheter can be conveniently introduced into a

5   femoral artery and threaded retrograde through the iliac artery and abdominal aorta and into a coronary artery. Alternatively, a catheter can be first introduced into a brachial or carotid artery and threaded retrograde to a coronary artery. Detailed descriptions of these and other techniques can be found in the art (see, e.g., above, including: Topol, (ed.), The Textbook of Interventional Cardiology, 4th Ed.

10   (Elsevier 2002); Rutherford, Vascular Surgery, 5th Ed. (W. B. Saunders Co. 2000); Wyngaarden et al. (eds.), The Cecil Textbook of Medicine, 22nd Ed. (W. B. Saunders, 2001); and Sabiston, The Textbook of Surgery, 16th Ed. (Elsevier 2000)).

By way of illustration, liposomes and other lipid-containing gene delivery complexes can be used to deliver one or more transgenes. The principles of the

15   preparation and use of such complexes for gene delivery have been described in the art (see, e.g., Ledley, Human Gene Therapy, 6:1129 (1995); Miller et al., FASEB Journal, 9:190 (1995); Chonn et al., Curr. Opin. Biotech., 6:698 (1995); Schofield et al., British Med. Bull., 51:56 (1995); Brigham et al., J. Liposome Res., 3:31 (1993)).

Administration of the gene therapy vector in accordance with the present

20   invention may be continuous or intermittent, depending, for example, upon the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of the gene therapy vector may be essentially continuous over a preselected period of time or may be in a series of spaced doses. Both local and

25   systemic administration is contemplated.

One or more suitable unit dosage forms comprising the gene therapy vector, which may optionally be formulated for sustained release, can be administered by a variety of routes including oral, or parenteral, including by rectal, buccal, vaginal and sublingual, transdermal, subcutaneous, intravenous, intramuscular,

30   intraperitoneal, intrathoracic, intrapulmonary and intranasal routes. The formulations may, where appropriate, be conveniently presented in discrete unit

dosage forms and may be prepared by any of the methods well known to pharmacy. Such methods may include the step of bringing into association the vector with liquid carriers, solid matrices, semi-solid carriers, finely divided solid carriers or combinations thereof, and then, if necessary, introducing or shaping the product into

5 the desired delivery system.

Pharmaceutical formulations containing the gene therapy vector can be prepared by procedures known in the art using well known and readily available ingredients. For example, the agent can be formulated with common excipients, diluents, or carriers, and formed into tablets, capsules, suspensions, powders, and

10 the like. The vectors of the invention can also be formulated as elixirs or solutions for convenient oral administration or as solutions appropriate for parenteral administration, for instance by intramuscular, subcutaneous or intravenous routes.

The pharmaceutical formulations of the vectors can also take the form of an aqueous or anhydrous solution or dispersion, or alternatively the form of an

15 emulsion or suspension.

Thus, the vector may be formulated for parenteral administration (e.g., by injection, for example, bolus injection or continuous infusion) and may be presented in unit dose form in ampules, pre-filled syringes, small volume infusion containers or in multi-dose containers with an added preservative. The active ingredients may

20 take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredients may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilization from solution, for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water, before use.

25 These formulations can contain pharmaceutically acceptable vehicles and adjuvants which are well known in the prior art. It is possible, for example, to prepare solutions using one or more organic solvent(s) that is/are acceptable from the physiological standpoint.

For administration to the upper (nasal) or lower respiratory tract by

30 inhalation, the vector is conveniently delivered from an insufflator, nebulizer or a pressurized pack or other convenient means of delivering an aerosol spray.

Pressurized packs may comprise a suitable propellant such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount.

5   Alternatively, for administration by inhalation or insufflation, the composition may take the form of a dry powder, for example, a powder mix of the therapeutic agent and a suitable powder base such as lactose or starch. The powder composition may be presented in unit dosage form in, for example, capsules or cartridges, or, e.g., gelatine or blister packs from which the powder may be

10 administered with the aid of an inhalator, insufflator or a metered-dose inhaler.

For intra-nasal administration, the vector may be administered via nose drops, a liquid spray, such as via a plastic bottle atomizer or metered-dose inhaler. Typical of atomizers are the Mistometer (Wintrop) and the Medihaler (Riker).

The local delivery of the vectors can also be by a variety of techniques

15 which administer the vector at or near the site of disease. Examples of site-specific or targeted local delivery techniques are not intended to be limiting but to be illustrative of the techniques available. Examples include local delivery catheters, such as an infusion or indwelling catheter, e.g., a needle infusion catheter, shunts and stents or other implantable devices, site specific carriers, direct injection, or

20 direct applications.

For topical administration, the vectors may be formulated as is known in the art for direct application to a target area. Conventional forms for this purpose include wound dressings, coated bandages or other polymer coverings, ointments, creams, lotions, pastes, jellies, sprays, and aerosols, as well as in toothpaste and

25 mouthwash, or by other suitable forms. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base and will in general also contain one or more emulsifying agents, stabilizing agents, dispersing agents, suspending agents, thickening agents, or coloring agents. The

30 active ingredients can also be delivered via iontophoresis, e.g., as disclosed in U.S. Patent Nos. 4,140,122; 4,383,529; or 4,051,842. The percent by weight of a

therapeutic agent of the invention present in a topical formulation will depend on various factors, but generally will be from 0.01% to 95% of the total weight of the formulation, and typically 0.1-25% by weight.

When desired, the above-described formulations can be adapted to give sustained release of the active ingredient employed, e.g., by combination with certain hydrophilic polymer matrices, e.g., comprising natural gels, synthetic polymer gels or mixtures thereof.

Drops, such as eye drops or nose drops, may be formulated with an aqueous or non-aqueous base also comprising one or more dispersing agents, solubilizing agents or suspending agents. Liquid sprays are conveniently delivered from pressurized packs. Drops can be delivered via a simple eye dropper-capped bottle, or via a plastic bottle adapted to deliver liquid contents dropwise, via a specially shaped closure.

The vector may further be formulated for topical administration in the mouth or throat. For example, the active ingredients may be formulated as a lozenge further comprising a flavored base, usually sucrose and acacia or tragacanth; pastilles comprising the composition in an inert base such as gelatin and glycerin or sucrose and acacia; mouthwashes comprising the composition of the present invention in a suitable liquid carrier; and pastes and gels, e.g., toothpastes or gels, comprising the composition of the invention.

The formulations and compositions described herein may also contain other ingredients such as antimicrobial agents or preservatives.

Exemplary Vectors for Selected Conditions

In one embodiment, to inhibit or treat chronic heart failure (CHF), vectors are employed that encode gene products that improve calcium homeostatis and β-adenoreceptor function in cardiomyocytes. For instance, in one embodiment, to prevent, inhibit or treat CHF, a gene therapy vector, e.g., an adenoviral vector, which contains a Serca2A, phospholamban, β-AR, β-ARK1 inhibitor, V2 vasopressin receptor, or adenylyl cyclase-type VI gene operably linked to regulatable promoter is employed. The expression of such a gene in a mammal having CHF can enhance LV function, e.g., LV contactability. For example, to treat

heart failure, a gene therapy vector having a regulatable transcriptional control element operably linked to an open reading frame for Serca2A is employed. In one embodiment, the regulatable transcriptional control element is regulated by a light sensitive promoter. After the gene therapy vector is administered to a mammal and

5 an implanted device detects reduced cardiac function, e.g., decreased HRV, the device emits light of a wavelength or energy that activates the regulatable transcriptional control element in the gene therapy vector. Serca2A expression is then upregulated in an amount effective to increase cardiac performance. To inhibit apoptosis which may contribute to loss of cardiomyocytes and cardiac function in

10 CHF, Bcl-2, Akt (protein kinase B), or phosphatidylionsitol-3 kinase may be employed in the same or different gene therapy vectors.

In another embodiment, to prevent, inhibit or treat atrial fibrillation, vectors are employed that contain genes for HSP, cardiomyocyte dedifferentiation, *mink*, a connexin, e.g., connexin 40, Serca2A, ryanodine receptor, proBNP, NPR-A, or one

15 of Kir2.1-2.4. In particular, the inward rectifier potassium current (I_{K1}) is notable for its intense expression in electrically quiescent atria and ventricle, but not in nodal pacemaker cells. I_{K1}, encoded by the Kir2 gene family, stabilizes a strongly negative resting potential and may suppress excitability.

Thus, one embodiment, to treat atrial fibrillation, a gene therapy vector

20 having a thermal sensitive transcriptional control element is linked to an open reading frame which encodes a gene product which alters the amount or activity of I_{K1}. After the gene therapy vector is administered to a mammal and an implanted device detects atrial fibrillation, e.g., by an electrogram, the device emits a thermal signal so as to increase expression of the gene product in an amount effective to

25 alter the amount or activity of I_{K1}. After a predetermined time, or when the device detects the absence of atrial fibrillation, the thermal signal is terminated.

All publications, patents and patent applications are incorporated herein by

30 reference. While in the foregoing specification, this invention has been described in relation to certain preferred embodiments thereof, and many details have been set

forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details herein may be varied considerably without departing from the basic principles of the invention.